The Journal of Laboratory and Clinical Medicine

VOL. 26 DECEMBER, 1940 NO. 3

PROGRESS

A CRITICAL HISTOPATHOLOGIC STUDY*

FIFTY POST-MORTEM PATIENTS WITH CANCER SUBJECTED TO LOCAL OR GENERALIZED REFRIGERATION COMPARED TO A SIMILAR CONTROL GROUP OF 37 NONREFRIGERATED PATIENTS

MACHTELD E. SANO, M.D., AND LAWRENCE W. SMITH, M.D.
PHILADELPHIA, PA.

IN PREVIOUS reports[1] the several members of our group who have been working upon the problems of reduced temperatures as applied particularly to cancer and embryonal cell growth have recorded their preliminary observations and deductions in rather broad generalities. This has been done intentionally in order to arouse interest in hypothermy both as a possible therapeutic agent and as a new approach experimentally to our understanding of human physiology.

The purpose of this paper is to reinforce these earlier studies by a more detailed analysis of the pathologic material obtained from the first 50 cancer patients who had undergone varying periods of refrigeration, either local, general, or combined, and who subsequently died. As a control, a similar series of 37 patients with cancer who died without having received refrigeration has been studied comparably. In this latter group the only selective factor which has entered the picture has been an effort to choose persons with the same primary organ involvement as those in the refrigeration series.

As it works out the refrigeration group tended to include, as might be expected, more advanced cases. The autopsies were all done by the same staff members, so that the two groups are strictly comparable in respect to the interpretation of any abnormal findings. We are not concerned in this paper

*From the Departments of Pathology, Temple University School of Medicine and Hospital, Philadelphia.

Aided by a grant from the Clinical Research Foundation, Philadelphia.

with any of the clinical aspects of the cases other than statistical; i.e., age of patient, duration and extent of disease, location of primary tumor, former treatment, whether surgical, radiologic, or combined, and the time relationship of such treatments, recurrence, metastases, etc. From the standpoint of refrigeration, ideally we should like to collect sufficient data to be able to tell whether continuous application is of greater effect than when refrigeration is given on the divided or fractionated dose principle, whether it is the *total* number of hours or the depth to which the temperature is successfully lowered and maintained, or some combination of these various procedures, which is the most successful in producing regressive cell changes in tumor tissue. As it is, in a small series of this size the most we can hope to do is to divide the cases on the basis of total number of hours of refrigeration received.

Since the publication of our initial studies concerning the effect of cold upon cancer and embryonal cell growth, a very considerable amount of discussion has resulted. One of the more critical of the statements has intimated that the growth of cancer cells is not only *not* impaired by cold, but is even *accelerated* and results in a wider dissemination of metastases. True, the observations are based on animal experiments[2] rather than on clinical studies. However, we feel that it is most important to know whether such acceleration of growth does occur in human beings or to what degree it is indicated, as upon such information depends the clinical selection and conduct of our cases. It is to study such questions that the careful and critical analysis of these autopsied cases seems so important to us, groping as we are in a field which is as yet totally unexplored.

Even though time may prove that such acceleration of cell growth may occur ultimately with refrigeration, it will not exclude refrigeration as a possible therapeutic weapon any more than it will exclude x-ray or surgery in which such effects are well recognized as occurring at times. The most pessimistic among us cannot deny that hypothermy has helped solve the problem of intractable pain in our patients with cancer. Any therapeutic agent must be weighed seriously as to its relative advantages and disadvantages before it is either used or discarded. It is from this purely objective standpoint that we wish to present in tabular form the histopathologic findings in these initial 50 refrigeration patients who were autopsied and compare them with a similar nonrefrigerated series.

The patients fell into three major groups, and a fourth group made up of miscellaneous forms of cancer. The three principal groups were (1) mammary, (2) uterine-cervical, and (3) gastrointestinal carcinoma. The fourth group was composed of tumors from almost every other part of the body, including lung, liver, adrenal, bladder, prostate, testis, mouth, thyroid, bone, lymph nodes and brain, as well as two cases of acute leucemia.

Fifteen of the 50 patients received local refrigeration treatment only, and properly might be excluded from the series as a whole. The great majority of the patients received less than 150 hours of generalized refrigeration (i. e., 30 persons, or 60 per cent), the time ranging from 24 to 150 hours. Only 5 persons received more than 150 hours of general hypothermy in this earlier series, although in subsequent studies more than twice this time interval was

not infrequently employed. It should be noted also, that in this initial series of cases the temperature level in general was maintained between 86° and 90° F., whereas in the later cases temperatures of approximately 8° to 10° lower were employed. Thus, the further analysis of a larger series of cases studied in the lower temperature range and maintained at these lower levels for much longer periods of time may require the revision of our figures and lead to other interpretations than are possible with these data.

TABLE I

MAMMARY CANCER—24 PATIENTS

ORGAN	LESION	REFRIGERATION GROUP		NONREFRIGERATION GROUP		HENKE AND LUBARSCH (%)	EWING 423 PATIENTS (%)
		NO. PATIENTS	%	NO. PATIENTS	%		
Heart	Normal	1	8.3	1	8.3		
	Myocardosis I and II	8	66.6	4	33.3		
	Myocardosis III and IV	1	8.3	7	58.1		
	Myocardial edema	2	16.6	3	25.0		
	Metastases	2	16.6	3	25.0	5	
	Pericarditis	1	8.3	0	0		
Lung	Bronchopneumonia	7	58.3	4	33.3		
	Metastases	12	100.0	9	75.0	50	49.9
Liver	Degenerations	7	58.1	5	41.5		
	Metastases	8	66.6	8	66.6	48-63	48.6
Spleen	Metastases	0	0	1	8.3	19	7
Kidneys	Toxic nephrosis	4	33.3	2	16.6		
	Metastases	2	16.6	2	16.6	5-40	
Pancreas	Pancreatitis	1	8.3	0	0		
	Metastases	1	8.3	1	8.3	Rare	
Adrenals	Medullosis	2	16.6	1	8.3		
	Metastases	4	33.3	5	41.5	27-56	
G. I. tract	Metastases	0	0	1	8.3	Rare	
Uterus	Metastases	2	16.6	2	16.6	5	
Ovary	Metastases	5	41.5	None noted grossly		8	
Thyroid	Metastases	2	16.6	None noted grossly		Rare	
Pituitary	Metastases	1	8.3	None noted grossly		Rare	
Skeleton	Vertebral metastases	12	100.0	Only 4 cases recorded but examination incomplete		19-80	20
	Other bones	2	16.6				
Brain and dura	Metastases	4	33.3	None noted clinically		3-19	9.5
	Edema	3	25.0	None examined			

With these preliminary comments we will present the statistical data in tabular form, comparatively arranged in the several groups for convenience. In Table I 24 cases of advanced breast cancer are shown. These were chiefly of the "simplex" type and varied from extremely cellular, medullary lesions to equally extreme scirrhous cases. The age of the patients ranged from 32 to 56 years, those in the lower age group predominating. Of the 12 patients subjected to refrigeration, all but one received generalized hypothermy, this one received cold locally. The 12 control patients received the usual suppor-

tive and symptomatic treatment. Several instances of operative and radiologic intervention are included in this latter group. Each of the persons who was refrigerated came to the hospital because of intractable pain, after all possible surgical and irradiation therapy had been employed, and after opiates had become ineffective. The duration of the generalized refrigeration varied from 50 to 230 hours, and the temperature range was from 83° to 90° F, the average being about 87° F.

Table I gives us a certain amount of information, much of it negative in character, but nevertheless of some importance and significance in our efforts to evaluate the effect of reduced body temperatures upon the activity of cancerous cells. In the first place, it is obvious that the entire 24 patients represent a much more advanced stage of malignancy than the average admission group, as shown by the widespread metastases in comparison with the figures from world literature compiled by Henke and Lubarsch in their *Handbook of Pathology*, and the series of 423 cases of breast cancer analyzed by Ewing. The metastatic figure is even greater in the 12 persons given refrigeration than in the corresponding 12 control patients, showing 100 per cent lung and skeleton involvement as compared with 75 and 33 per cent, respectively. Furthermore, these metastases were all present at the time of admission. Comment upon their serial roentgen-ray studies will be made later in this discussion in relation to the entire 50 cases. Suffice it to say that from the data available, we have no evidence to suggest that the rate of growth of such metastatic foci is accelerated. On the contrary, in some few instances it actually seems retarded. However, in view of the advanced stage of the disease in this group of patients as a whole, it is not possible either to prove or to disprove whether hibernation does or does not increase the spread of metastases or even alter the rate of growth of such metastatic tissue within the relatively short span of life during which they were under observation.

TABLE II

RELATIONSHIP OF METASTASES (PULMONARY) TO DEVELOPMENT OF BRONCHOPNEUMONIA IN 24 PATIENTS WITH BREAST CANCER

GROUP	PATIENTS WITH METASTASES					PATIENTS WITH NO METASTASES				
	NO. WITH METASTASES	BRONCHOPNEUMONIA		NO PNEUMONIA		NO. WITHOUT METASTASES	BRONCHOPNEUMONIA		NO PNEUMONIA	
		NO.	%	NO.	%		NO.	%	NO.	%
Refrigerated	11	7	58.3	5	41.7	0	0	0	0	0
Nonrefrigerated	9	3	33.3	6	67.0	3	1	33.3	2	67

Aside from the question of metastases two other features of interest pathologically have been noted which relate (1) to the part played by the heart during refrigeration and (2) the incidence and importance of bronchopneumonia comparatively in the refrigerated and nonrefrigerated cases. Both of these questions will be discussed in greater detail later, in respect to the entire 87 cases. So far as myocardial failure is concerned there seems to be no significant difference between the two groups.

The incidence of bronchopneumonia requires more detailed analysis. There is one factor which seems possibly significant in influencing the occurrence of pneumonia in both the refrigerated and nonrefrigerated groups, viz., the

presence or absence of metastases. The series of patients with cancerous breast seem to be representative of this point, having a particularly high incidence of metastases because of their anatomic relationship. In Table II the data are presented with this in mind, and an attempt is made to correct statistically for the difference in pulmonary metastatic incidence in the two groups.

From these figures it can be stated that the presence of pulmonary metastases, as might well be anticipated, is apparently associated with a somewhat higher incidence of terminal bronchopneumonia in the refrigerated series of persons with breast cancer. It might be added that under such metastatic conditions, the pneumonic incidence appears to be somewhat greater in the cases maintained under generalized refrigeration for the more prolonged periods of time. In a series of cases as small as this we are not certain whether the differences are of such magnitude as to be statistically significant, but we have gained the impression that where such pulmonary metastases do exist one must recognize an added risk in recommending generalized refrigeration for any given case.

TABLE III

CERVICAL CANCER—10 PATIENTS

ORGAN	LESION	REFRIGERATION GROUP		NONREFRIGERATION GROUP		HENKE AND LUBARSCH (%)	OTHER AUTHORS (%)
		NO. PATIENTS	%	NO. PATIENTS	%		
Heart	Normal	1	20	0	0		
	Myocardosis I and II	4	80	4	80		
	Myocardosis III and IV	0	0	1	20		
	Metastasis	0	0	0	0		
Lung	Bronchopneumonia	2	40	3	60		
	Metastasis	1	20	1	20		5
Liver	Toxic changes	4	80	2	40		
	Metastasis	0	0	2	40	15	
Spleen	Toxic changes	4	80	2	40		
	Metastasis	0	0	0	0		
Kidney	Pyonephrosis	1	20	1	20		
	Toxic nephrosis	3	60	2	40		
	Metastases	0	0	0	0		
Bladder	Metastatic extension	2	40	1	20		
Pancreas	Pancreatitis	1	20	0	0		
	Metastasis	0	0	1	20		
Adrenal	Metastasis	0	0	1	20		
Intestine	Metastasis	1	20	0	0		
Uterus	Metastatic extension	2	40	4	80		14-50 (Sheib, Schottlander)
Ovary	Metastasis	0	0	0	0	Rare	
Brain	Metastasis	0	0	No post-mortem examination made			
	Edema	2	40				
Skeleton	Metastasis	0	0	0	0		

The second group of patients, somewhat smaller in number, who had advanced squamous carcinoma of the cervix uteri, are analyzed in Table III in the same way as the patients with cancer of the breast were presented. Of

the five patients who received refrigeration, only three were given cold locally, while the other two required generalized refrigeration to control the pain. As might be expected, the incidence of metastases in this group of cases was much less than in the breast cases, and inasmuch as cold could be applied successfully locally at a much lower temperature range than that obtained by the generalized reduction of body temperature, it was the method of choice.

A series of ten patients with cervical carcinoma, analyzed in Table III, presents a totally different clinical problem than the patients with breast cancer. The disease is a local one, with regional extension of the tumor rather than distant metastasis. In only two cases were pulmonary metastases found, and in neither of these was pneumonia present terminally.

The third group, consisting of twelve persons with carcinoma of gastrointestinal tract, do not parallel each other quite as closely as the previous series, there being a slight preponderance of gastric cases in the nonrefrigerated series. Of the remaining five experimental cases, two were primary in the transverse colon, one in the sigmoid, one in the rectum, and one represented a peptic ulcer just below the pyloric ring which had undergone malignant degeneration and showed widespread metastasis. The analysis of this series is presented in Table IV.

TABLE IV

GASTROINTESTINAL TRACT CARCINOMA—12 PATIENTS

ORGAN	LESION	REFRIGERATION GROUP		NON-REFRIGERATION GROUP		HENKE AND LUBARSCH (%)	OTHER AUTHORS (%)
		NO. PATIENTS	%	NO. PATIENTS	%		
Heart	Normal	0	0	0	0		
	Myocardosis I and II	3	50	5	83.5		
	Myocardosis III and IV	3	50	1	16.5	0.5	
	Metastasis	0	0	0	0		
Lung	Bronchopneumonia	4	66.6	3	50.0		
	Lobar pneumonia	0	0	1	16.5		
	Empyema	1	16.5	0	0		
	Metastasis	2	33.3	2	33.3	4-13	
Liver	Toxic changes	3	50	1	16.5		
	Metastasis	4	66.6	2	33.3	45-56	34
Spleen	Toxic changes	1	16.5	0	0		
	Metastasis	1	16.5	0	0	3.4-6.5	8.3
Kidneys	Toxic changes	1	16.5	2	33.3		
	Pyonephrosis	1	16.5	0	0		
	Metastasis	1	16.5	0	0	1.3-4.4	13
Bladder	Metastatic invasion	1	16.5	0	0	1.1	
Pancreas	Pancreatitis	1	16.5	0	0		
	Metastasis	2	33.3	0	0	3.3	
Adrenals	Adenoma	2	33.3	0	0		
	Metastasis	2	33.3	0	0	1.3-7.6	6
Uterus	Metastatic invasion	1	16.5	0	0	0.5	
Ovaries	Metastasis	0	0	0	0		
Prostate	Metastasis	1	16.5	0	0	0.1	
Thymus	Persistence	1	16.5	0	0		
Brain	Edema	1	16.5	0	0		
	Metastasis	0	0	0	0		
Skeleton	Metastasis	0	0	0	0		

In this group of gastrointestinal cases of malignancy, all the experimental cases received both local and general refrigeration. The time of the latter ranged from 150 to 230 hours, a distinctly more prolonged technique on the average than that used in the breast group. Yet when we look at the figures relating to the incidence of bronchopneumonia in these cases, there is no significant difference between the refrigerated and the nonrefrigerated cases. Even the presence of lung metastases does not seem to be an unduly important factor in determining this complication, as less than half the cases in the series of cervical and gastrointestinal carcinoma with lung metastases demonstrated any evidence of bronchopneumonia at autopsy.

Another point to which attention should be called is the curious finding of occasional cases of acute pancreatic degeneration microscopically. This may be accompanied by a small amount of fat necrosis and hemorrhage, and thus become a grossly demonstrable process in rare instances, as has been observed in more recent cases than those subjected to analysis in this early series. We are inclined to feel that pancreatitis is another possible complication of generalized refrigeration. On the basis of Oppenheimer and McCravey's[3] studies, which established the fact that circulation time is definitely slowed, this can be explained perhaps as being the result of tissue anoxia, thus permitting the escape and activation of the pancreatic enzymes. We are of the opinion, however, that careful following of the blood amylase of patients during "hibernation" precludes serious danger.

TABLE V

27 PATIENTS WITH MISCELLANEOUS MALIGNANCY SUBJECTED TO REFRIGERATION

CASE NO.	SITE OF PRIMARY TUMOR	DIAGNOSIS	REFRIGERATION PERIOD	
			GENERAL (HR.)	LOCAL
1	Adrenal	Hypernephroma, malignant	–	5 weeks
2	Antrum	Carcinoma, squamous cell	–	12 weeks
3	Antrum	Carcinoma, squamous cell	–	10 days
4	Auditory canal	Carcinoma, squamous cell	–	3 weeks
5	Bladder	Carcinoma, squamous cell	48	12 weeks
6	Bladder	Carcinoma, squamous cell	–	16 weeks
7	Blood	Leucemia, acute lymphatic	90	–
8	Blood	Leucemia, acute lymphatic	230	–
9	Bone (tibia)	Osteogenic sarcoma	90	–
10	Bone (sacrum)	Fibromyxosarcoma	–	200 days
11	Brain	Glioblastoma multiforme	–	3 weeks
12	Liver	Hepatoma malignum	90	–
13	Lung	Bronchiogenic carcinoma	24	3 days
14	Lung	Bronchiogenic carcinoma	90	1 day
15	Lymph node	Hodgkin's disease	120	–
16	Mediastinum	Lymphosarcoma	50	–
17	Pancreas	Adenocarcinoma—duct type	50	–
18	Prostate	Adenocarcinoma	–	6 weeks
19	Retroperitoneum	Fibromyxosarcoma	120	–
20	Retroperitoneum	Reticulo-cell sarcoma	24	–
21	Skin	Melanoma, amelanotic, malignum	120	–
22	Submaxillary	Adenocarcinoma	–	30 weeks
23	Testis	Choriocarcinoma	–	1 week
24	Thyroid	Adenocarcinoma	120	–
25	Tongue	Carcinoma, squamous cell	50	–
26	Tonsil	Carcinoma, squamous cell	24	–
27	Uterus	Adenocarcinoma—fundus	–	3 weeks

In presenting the remaining 27 cases, the data have been condensed so far as possible into comparable tabular form as in the previous groups. The cases, however, as shown in Table V, are almost entirely single instances of a very wide variety of neoplasms, selected in part on that very basis in order to have the opportunity of studying the effect of reduced temperatures upon as many types of malignant cells as possible. For that reason, it is obviously impossible to present statistical data in respect to any one of these various forms of malignant disease individually, but the composite figures appear to fit in with those of the three other series already presented.

DISCUSSION

At this point it seems well to recapitulate the major pathologic findings which this survey has disclosed, and to try to compare them in the refrigerated and nonrefrigerated series of patients. As has been commented upon in earlier papers, the persons who have been observed under refrigeration in this study were all advanced, terminal examples of malignancy. None of them, therefore, have had a survival period long enough to be of much significance in respect to the question of the effect of cold upon metastatic foci. The interest centers chiefly upon the immediate effects of cold on neoplastic cell growth and on various physiologic mechanisms, as evidenced by demonstrable histopathologic changes in the tissues of the several organ systems. To this end let us attempt to discuss the findings separately in those organs where such demonstrable pathology has been found to exist.

Heart.—As has been shown by others,[4] as well as by our own studies,[5, 6] generalized reduction of body temperature is quite regularly accompanied by alterations in the electrocardiogram which suggest rather widespread myocardial involvement; i.e., a depression of the T-wave and prolongation of the Q-S interval.

Furthermore, occasional instances of sudden fall in blood pressure followed by heart failure, and associated with very acute myocardial degeneration at autopsy, led us to suspect that the slowed circulation might present a serious problem insofar as maintenance of the nutrition of the heart muscle was concerned. Analysis of the autopsy material in these cases under consideration has been somewhat reassuring in this respect. In the 50 patients subjected to refrigeration a total of 42 (84 per cent) have shown some degree of myocardial degeneration, by far the greater number of grades I and II. On the other hand, 35 (94 per cent) of the 37 control patients have shown similar changes, with a definitely higher proportion in the grades III and IV classification comparatively.

It is doubtful whether any real importance should be placed on these relatively minor differences of percentage, although the grading in each instance was done by three members of the department independently with only very few and minor discrepancies. Thus, it seems fair to state that there is no apparent or significant difference in the actual incidence of myocardial degeneration in the two groups.

Respiratory Tract.—In attempting to evaluate the data relating to the respiratory tract, one problem, that of the incidence and relationship of broncho-

pneumonia to refrigeration, is obviously paramount. We have already seen in presenting the figures from the group of patients with breast cancer that the existence of pulmonary metastases seemed to increase the likelihood of the development of a bronchopneumonia during generalized refrigeration. However, in the comparable series of cases of breast cancer in the nonrefrigerated group the percentage incidence of terminal bronchopneumonia was identical (67 per cent) in the metastatic and nonmetastatic cases.

TABLE VI

COMPARATIVE FINDINGS (A) IN 41 PATIENTS WITH MISCELLANEOUS MALIGNANCY AND (B) IN ENTIRE COMBINED GROUPS—91 PATIENTS

ORGAN	LESION	(A)				(B)			
		REFRIGERATED GROUP (27 PATIENTS)		NON-REFRIGERATED GROUP (14 PATIENTS)		REFRIGERATED GROUP (50 PATIENTS)		NON-REFRIGERATED GROUP (37 PATIENTS)	
		NO. PATIENTS	%	NO. PATIENTS	%	NO. PATIENTS	%	NO. PATIENTS	%
Heart	Normal	4	15.0	1	7.1	6	12.0	2	5.4
	Myocardosis I and II	19	70.0	10	71.4	34	68.0	23	62.1
	Myocardosis III and IV	4	15.0	3	21.4	8	16.0	12	32.4
	Metastasis	5	18.5	3	21.4	5	10.0	6	16.2
Lungs	Bronchopneumonia	18	66.6	9	64.2	30	60.0	19	51.3
	Lobar pneumonia	1	3.0	0	–	1	2.0	1	2.7
	Metastasis	9	33.3	5	35.7	24	48.0	17	45.9
Liver	Toxic changes	17	63.0	7	50.0	31	62.0	15	40.5
	Metastasis	7	26.0	5	35.7	19	38.0	17	45.9
Spleen	Toxic changes	10	37.0	6	42.8	15	30.0	8	21.6
	Metastasis	4	15.0	4	28.5	5	10.0	5	13.5
Kidneys	Toxic nephrosis	14	51.0	7	50.0	22	44.0	12	32.4
	Pyonephrosis	3	11.0	4	28.5	5	10.0	5	13.5
	Metastasis	6	22.0	2	14.2	9	18.0	4	10.8
Bladder	Metastatic extension	3	11.0	1	7.1	6	12.0	2	5.4
Pancreas	Pancreatitis	2	7.4	0	–	5	10.0	0	–
	Metastasis	5	18.5	1	7.1	8	16.0	3	8.1
Adrenals	Toxic changes	5	18.5	–	–	7	14.0	–	–
	Metastasis	6	22.0	4	28.5	12	24.0	10	27.0
G. I. Tract	Metastasis	5	18.5	4	28.5	6	12.0	5	13.5
Uterus	Metastasis	2	7.4	1	7.1	7	14.0	7	18.9
Ovary	Metastasis	2	7.4	1	7.1	7	14.0	5	13.5
Brain	Edema	10	37.0	3	21.4	16	32.0	–	–
	Metastasis	3	11.0	0	–	7	14.0	3	8.1
Skeleton	Metastasis	7	26.0	3	21.4	19	38.0	7	18.9
Thyroid	Metastasis	2	7.4	0	–	4	8.0	0	–

When we turn to a consideration of both entire series, including the patients with cancer of the breast, as is shown in Table VI(B), we find the total incidence of bronchopneumonia is practically the same; 60 per cent in the refrigerated group and 53 per cent in the nonrefrigerated group. It is interesting to try to break down these figures in the refrigerated group in respect both to the presence of metastases and to the amount of generalized refrigeration the patients received.

What conclusions, if any, can be drawn from these figures in respect to the relationship of refrigeration and pneumonia? We have seen from the control patients that there is a normal expectancy of at least 50 to 60 per

cent of pneumonia in terminal malignancy, and that there is no appreciable difference in incidence between the persons with and without pulmonary metastases.

In the experimental series of 50 cases there occurred a total of 32 cases of bronchopneumonia. However, we must exclude those cases which occurred in patients who received only local refrigeration, i.e., 9 cases, as these cases could more properly be included in the control group. It is interesting to note that of the 15 patients treated only locally the incidence of pneumonia remains at the 60 per cent average level.

Of the 23 cases of pneumonia which occurred in patients who underwent "hibernation," 13 (56 per cent), or only slightly over half showed the presence of metastases. If we exclude the 7 patients with breast cancer who had pneumonia, we find that only 6 of the remaining cases (or 37 per cent) had metastases. In comparing these several figures analyzed variously, one can only come to the conclusion that the presence of pulmonary metastases does not significantly change the incidence of bronchopneumonia among patients undergoing generalized refrigeration. Likewise (and this is the point which above all we have been interested in determining), if these statistics can be accepted as of real significance, then they all point inevitably to the conclusion that generalized reduction of body temperature ("hibernation") does not change the incidence of terminal pneumonia in patients suffering from advanced malignant disease.

Liver, Spleen, and Kidneys.—In grouping these abdominal viscera together for consideration, we are prompted by the fact that such changes as have been noted have all been strictly comparable. There appears to be a slightly higher incidence of the so-called "toxic" degenerative reversible type of reaction observed in the refrigerated group than in the nonrefrigerated group. In other words, the mild, nonspecific granular changes seen so commonly in the liver and kidneys have been noted about 10 to 15 per cent more frequently in patients who have undergone "hibernation" than in those who have not. However, one must take into consideration the pneumonic incidence in relation to these changes, the amount of absorption from degenerating tumor tissue, the presence of metastases, and so many other variable factors that it seems only reasonable to assume that such minor changes as have been noted are within normal limits of variability. Certainly they do not appear to be sufficiently marked to be of any clinical importance.

Pancreas.—In the pancreas, on the other hand, it is our impression that we have pathologic changes among the refrigerated group which do not have their counterpart in the nonrefrigerated group. We might add that subsequent cases have given additional support to this contribution. While the actual incidence of pancreatic degenerative and inflammatory changes is only 10 per cent, it seems significant that *no* such changes have been found in the control group. These changes range from grossly apparent foci of fat necrosis or hemorrhage or both, to similar lesions of much less magnitude, demonstrable only microscopically. In line with Rich and Duff's[7] conclusions, and more recently those of Smyth,[8] these instances of so-called pancreatitis seem to

offer further substantiation to the theory of a vascular or circulatory origin in the development of the degenerative process. We feel that the slowed rate of blood flow may well be instrumental in producing tissue anoxia, followed by the characteristic pathology of the parenchyma of the organ. It most certainly offers a new approach to the experimental study of the condition.

As has already been commented upon, the following of these patients during "hibernation" by daily blood amylase determinations, as suggested by our biochemist, Dr. R. H. Hamilton, is a prophylactic measure which we have carried out routinely in subsequent studies. We are sure that at least one incipient severe instance of pancreatitis was prevented by this means, the patient being taken out of refrigeration with the sudden rise of the blood amylase to 1,100 units and the symptoms subsequently subsiding with the return of the amylase to normal levels.

Adrenal Glands.—No comparable control figures are available in respect to the adrenal glands. In going over the gross autopsy findings seldom was any comment found about any pathologic changes in these organs. Unfortunately, as a result microscopic sections were available in only a few instances. In view of the tremendous importance which Selye[9] places upon the adrenal glands in the defense mechanism of the body, it is to be regretted that more detailed studies are impossible. Medullary depletion and cortical hyperplasia, as described by Selye, have certainly been observed in a number of the refrigerated cases, but irregularly. In any subsequent study more careful attention should and will be paid to these organs.

Brain.—Particular attention has been focused clinically upon the central nervous system of these patients during hibernation. The complete failure to recall anything over the entire period, the dulling of the sensorium, the slowing-up of speech and understanding, the complete mental recovery with relief of pain and headache, and not infrequently the almost euphoric state which has been noted, have all stimulated the keenest interest in neuropathologic studies.

Of the 50 patients coming to autopsy, permission was obtained to examine the brains of 44. Metastasis was found in only three cases; hence this factor does not enter prominently into the picture. Eleven cases should perhaps be excluded as the patients had received only local cold therapy. This still leaves 33 patients to be considered: 11 of these received less than 50 hours of general refrigeration, 17 received from 50 to 150 hours, and 5 had from 150 to 230 hours of "hibernation."

Outstanding as the only grossly demonstrable pathologic finding in the entire series was the presence of edema, which it must be admitted is often a very difficult thing to estimate. However, edema was recorded in 12 patients who had been refrigerated, and in an additional 4 who had received only local cold therapy. There is an interesting similarity in the percentage figures of the incidence of edema in (1) the group as a whole as shown in Table VI(B), i.e., 32 per cent; (2) 12 of the 35 persons refrigerated, i.e., 34 per cent; and (3) 4 of the 15 persons who were not refrigerated, i.e., 27 per cent.

From these figures it is apparent that generalized refrigeration in itself does not seem to be a significant factor in producing cerebral edema. The duration of the refrigeration period likewise does not appear to be significant in respect to either the incidence or the severity of the edema. In fact, the greater proportion of the cases were found in the less than 50 hour group.

Microscopic study has failed to reveal any other pathology. No changes affecting motor and sensory neurones or glial cells have been demonstrated. Nothing in the nature of an inflammatory encephalitic reaction has been noted. Indeed, both from clinical observation and from analysis of this pathologic material, we have been unable to date to demonstrate any harmful effects of refrigeration upon the central nervous system.

Metastases.—A perusal of the several tables reveals at a glance how far advanced the cases of malignancy in this series have been, for the percentage involvement, with the exception of the lungs, is considerably higher in each organ system than the figures given by most authors. Such a study further confirms the rather characteristic distribution of metastases in the various forms of cancer which have been encountered, but adds little else, in this respect, to our previous knowledge. From the standpoint of the effect of refrigeration upon these metastatic foci, we are scarcely warranted in hazarding an opinion, based on the data available in this material. Since most of the patients died, the duration of life was so seldom more than a few weeks that one would scarcely expect to find any convincing evidence of regression. Furthermore, it is impossible to have a truly representative base line—biopsied tissues—from the metastatic areas. Thus, changes which are demonstrable at autopsy either grossly or microscopically are not necessarily indicative of regression, the result of refrigeration. Only time and a sufficient number of cases can establish factual evidence in this regard.

It is true that regressive changes are likely to be observed in the metastatic tissues from the refrigerated group. It is equally true that similar changes may be observed in any malignant tumor. Undoubtedly, it is almost impossible to exclude bias entirely in a matter where one's interest and enthusiasm are so closely bound up. Nonetheless, it is our impression that in the majority of those patients subjected to more than 120 hours of "hibernation," the regressive cell changes seem to be more marked, and to occur with greater regularity in persons subjected to refrigeration than in those who were not. In no instance, however, has complete necrosis of any metastatic area been observed.

X-RAY STUDIES

The only possible way to evaluate accurately the gross regression of metastatic tumor is by means of serial x-ray studies where the size of the lesion can actually be measured at successive intervals. Through the splendid cooperation of the members of the Department of Radiology, especially Dr. W. E. Chamberlain, Dr. B. R. Young, and Dr. R. K. Arbuckle, such studies were undertaken whenever possible as a routine control yardstick for the

clinical and pathologic findings. Initial roentgenograms were obtained in 33 of the experimental group. In 14 of these no subsequent studies were obtained, in most cases because of the death of the patient before such repeat pictures were indicated. Of the 19 remaining patients, 9 must be eliminated because their treatment was by local cold application only. Of the remaining 10, three had less than 50 hours of refrigeration, and four others had from 50 to 150 hours; only three received over 150 hours of generalized refrigeration. No change was noted by x-ray in the group having less than 50 hours of refrigeration, nor in three who were in the second group (the 50 to 150 hour series); no apparent change was observed in one of the three (a case of breast cancer [E. B.] with widespread skeletal metastasis) who had as much as 230 hours of hibernation.

The other three cases perhaps merit individual comment. Case 15 (E. G.) was a patient with advanced breast cancer who received local treatment for several months, and then, in the terminal stages of her disease, was the first patient ever to undergo "hibernation." All that can be said is that during the last three months of her life there was no increase in the size of any of the skeletal metastases which in general had been progressive in character over the preceding six-month period.

Case 47 (A. W.) was a patient with carcinoma of the thyroid with widespread generalized and skeletal metastases, who was maintained under observation for about ten months. Most of his treatment was of the local type, but he did receive about 120 hours of generalized refrigeration. Following a three-month treatment period, the x-ray examination revealed definite improvement of many of the lesions, and the discovery of no new areas. Five months later improvement was still noted in the lesions in the cervical spine, the ribs and clavicle, but there was evidence of increased destruction in the pelvic bones.

Case 96 (L. Q.) was a patient who had the fulminating type of acute lymphatic leucemia that occurs in a child. He received no x-ray treatment other than that unavoidably required for repeated pictures. Over a two-month period he had several brief periods of refrigeration, following each of which there was a temporary measurable decrease in the mediastinal area of involvement, as well as of the spleen, accompanied by a profound drop in the white blood cell count. These regressive changes, however, were transitory and incomplete. Positive tissue cultures were obtained from cervical nodes at autopsy in spite of what grossly appeared to be complete liquefaction necrosis of the tissues.

Obviously, these data are totally inadequate with which to draw any conclusions in respect to the effects of generalized refrigeration upon metastatic tumor tissue. From such data as we do have it becomes apparent that no significant changes are likely to occur in much less than 240 hours of such low temperature application. Whether complete regression of such metastatic foci would ever result is extremely unlikely, but it does not seem unreasonable to feel that generalized hypothermy might well be an important adjunct to other forms of therapy. Its further exploration is urged.

SUMMARY

1. The histopathologic findings of 50 patients with malignant disease subjected to local, generalized, or combined refrigeration are compared to those of 37 closely analogous terminal cases of malignancy given only the usual supportive treatment during the terminal stages.

2. Critical analysis of the heart, lung, liver, spleen, and kidney findings reveals no very significant differences in the two groups.

3. The occurrence of acute pancreatic changes, either gross or microscopic, in about 10 per cent of the persons given refrigeration is particularly discussed from the standpoints of etiology and prevention.

4. The effect of refrigeration upon metastatic disease, as demonstrated by serial x-ray as well as autopsy studies, is discussed. It is suggested that at least 240 hours of such generalized refrigeration is apparently needed to induce any significant regression of such metastases, and that such regressions are of irregular occurrence only.

5. The further exploration of the use of reduced temperature as an adjunct to other forms of treatment of cancer is urged.

Acknowledgment should be made to a very considerable number of the hospital staff for their respective contributions in the conduct of these studies, but especially to Dr. Temple Fay and Dr. Augustus McCravey for their clinical observations; and to the members of the Pathology Department, Dr. E. E. Aegerter, Dr. H. C. Lennon, Dr. A. R. Peale, and Dr. L. L. Lawry, for their help in the performance of the autopsies. Dr. C. L. Brown as internist, Dr. W. E. Burnett as surgeon, Dr. H. Roesler as cardiologist, and Dr. G. C. Henny as physicist, likewise are particularly deserving of our gratitude.

REFERENCES

1. Fay, T., and Henny, G. C.: Correlation of Body Segmental Temperature and Its Relation to the Location of Carcinomatous Metastases, Surg. Gynec. & Obst. **66**: 512, 1938.
Smith, L. W., and Fay, T.: Temperature Factors in Cancer and Embryonal Cell Growth, J. A. M. A. **113**: 633, 1939.
2. Warren, Stafford L.: Personal communication.
3. Oppenheimer, N. J., and McCravey, A.: Circulation Time in Man at Low Temperatures, J. Physiol. **129**: 434, 1940.
4. Kossman, C.: General Crymotherapy, A Symposium: Cardiovascular Aspects, Bull. New York Acad. Med. **16**: 317, 1940.
5. Smith, L. W.: Refrigeration in Cancer—Pathological Observations, New York State J. Med. **40**: 1355, 1940.
6. Fay, T.: Observations in Prolonged Human Refrigeration, New York State J. Med. **40**: 1551, 1940.
7. Rich, A. R., and Duff, G. L.: Experimental and Pathological Studies on the Pathogenesis of Acute Hemorrhagic Pancreatitis, Bull. Johns Hopkins Hosp. **58**: 212, 1936.
8. Smyth, C. J.: Etiology of Acute Pancreatitis, Arch. Path. **30**: 651, 1940.
9. Selye, H., Dosne, Christianne, and Bassett, Luch: On the Therapeutic Value of Adrenal Cortical Hormones in Traumatic Shock and Allied Conditions, Canad. M. A. J. **43**: 1, 1940.

CLINICAL AND EXPERIMENTAL

THE DEVELOPMENT OF EOSINOPHILIA FOLLOWING LIVER THERAPY*

ROBIN N. ALLIN, M.D., AND OVID O. MEYER, M.D.
MADISON, WIS.

INTRODUCTION

SINCE the institution of liver in the treatment of pernicious anemia, it has been a well-known fact that eosinophilia sometimes develops following the administration of large amounts of raw or cooked whole liver. That eosinophilia may follow the oral administration of liver extract has remained a debated issue. A review of the literature has revealed no reference to the development of eosinophilia following the administration of parenteral liver extract.

In 1927 Watkins and Berglund[4] reported the development of a progressively rising eosinophilia in a patient with pernicious anemia. This patient had been placed on a raw liver diet. Up to the eighteenth day of observation the percentage of eosinophiles had remained relatively constant, but from that date on, frequent differential counts through the fiftieth day revealed a progressive eosinophilia to about 48 per cent. Whitby[6] in 1928 reported the development of eosinophilia in four cases of pernicious anemia following the institution of liver therapy. Two patients had received raw liver only. One patient had received a combination of raw and cooked liver, and one had received cooked liver only. Eight to eleven weeks after institution of therapy a rise in eosinophiles to 6 to 8 per cent was noted. In one patient receiving raw liver alone a maximum of 26 per cent eosinophiles was attained. Smith and Whitby[3] reported the occurrence of eosinophilia in patients convalescing from various medical and surgical conditions after they had received a diet of cooked liver over a period of from six to forty days. A maximum rise to 61 per cent was noted in one instance, but this was in a patient with Hodgkin's disease. Meulengracht and Holm[1] concluded that eosinophilia in the liver treatment of pernicious anemia appeared in a marked and persistent form when treatment was carried out with large doses of raw calves' liver and that this eosinophilia persisted as long as raw liver was given. The increase in eosinophiles in these cases ranged from 20 to 40 per cent, and in one case was as high as 74 per cent. Control patients suffering from various other diseases responded in a like manner. This eosinophilia was usually absent when fried liver or liver extract was employed. Whitby and Britton[5] hold to the view that eosinophilia occurs only with the administration of raw liver and not with liver extract. Murphy[2]

*From the Department of Medicine, University of Wisconsin, Madison.
This study has been aided in part by a grant from the Wisconsin Alumni Research Foundation.
Received for publication, November 15, 1939.

states that in patients ingesting either whole liver or liver extract there may occur an increase in the number of eosinophiles, in some cases to rather high levels. He reports one patient who received whole liver and in whom there developed an eosinophilia of 50 per cent of a total of 18,000 leucocytes. When this patient was given liver extract by mouth, there was some decrease, but not until the extract was given intramuscularly were normal values established.

REVIEW OF CASES OF PERNICIOUS ANEMIA SEEN IN THE STATE OF WISCONSIN GENERAL HOSPITAL

Since a review of the literature revealed few references to the development of eosinophilia following whole liver ingestion, no references to eosinophilia following parenteral liver therapy, and conflicting views regarding the development of eosinophilia following oral liver extract, it was felt that a study of the cases of pernicious anemia seen in the State of Wisconsin General Hospital from September, 1925, through September, 1938, might be instructive. This review was undertaken for the purpose of determining what form or forms of liver therapy produced eosinophilia, and to what extent.

TABLE I

ANALYSIS OF PERNICIOUS ANEMIA PATIENTS SEEN IN THE STATE OF WISCONSIN GENERAL HOSPITAL SEPTEMBER, 1925, THROUGH SEPTEMBER, 1938

TYPE OF THERAPY	TOTAL CASES	NUMBER WITH EOSINOPHILIA	PER CENT OF TOTAL WITH EOSINOPHILIA	PER CENT AVERAGE MAXIMUM EOSINOPHILIA	REMARKS
No specific treatment	45	24	53.3	5.9	Eosinophilia of unknown cause
Whole blood transfusions	9	2	22.2	20.8	Individual maximum eosinophilia 34.6% and 7.0%
Whole blood (intramuscular injections—5 c.c. twice a week)	1	1	100	30.0	Maximum eosinophilia obtained after 2 months
Whole cooked liver	13	10	76.9	8.6	
Extralin (Lilly)	1	1	100	8.0	
Cofron (Abbott)	1	1	100	10.0	
Oral liver extract No. 428—Lilly	2	0	0	0	
Oral liver extract No. 29—Valentine	7	3	42.8	6.0	
Oral liver extract No. 343—Lilly	30	5	16.6	6.8	
Mixed liver therapy	91	31	34.1	10.4	Various forms of oral and parenteral liver, individual maximum eosinophilia 50.0%
Intramuscular liver extract—Lilly	79	36	45.5	11.3	Individual maximum eosinophilia 45.0%
Total cases	279				
Total number of cases receiving liver					224
Total number of cases with eosinophilia after liver therapy (excluding 6 cases with allergic background)					87 (38.8%)

For the purpose of this study, as well as for the experimental work to be reported in this article, a percentage of eosinophiles in excess of four was considered abnormal.

A total of 279 cases of pernicious anemia was studied. Of these, 224 received some form or forms of liver therapy, and of this latter group 87 showed an eosinophilia at some time. The results are summarized in Table I.

As frequent differential counts were not routine, it was impossible to determine: (1) the interval between institution of liver therapy and the beginning of eosinophilia, and (2) whether or not there occurred a gradual progressive eosinophilia throughout the period of liver treatment or merely a sporadic increase in eosinophiles.

EXPERIMENTAL STUDIES

An effort was made to study more closely the eosinophilic response to various forms of liver treatment in well or nearly well individuals, and to compare this response to that seen in patients with pernicious anemia who had received no liver therapy and from whom no allergic history could be obtained.

METHODS

In studying the four control cases, seven different "regimens" were employed. Two patients received 0.5 c.c. of Lilly's reticulogen intramuscularly every other day for a total of seven injections. Two patients received 3.0 c.c. of Lilly's liver extract intramuscularly every other day for seven injections. Three patients received one-half pound of cooked whole beef liver daily for a period of fourteen days.

The patients with pernicious anemia were treated according to the regular schedule for uncomplicated pernicious anemia. They received 3.0 c.c. of Lilly's extract intramuscularly every fifth day throughout the entire period of their hospitalization.

All patients were followed for at least one month. Total leucocyte counts were made in duplicate. The percentage of eosinophiles was determined each time by counting a total of 400 cells on three cover slip smears stained by Kingsley's method. In all persons with pernicious anemia and in some of the controls counts were made daily. In no instance were counts made less than three times each week, and they were always made at approximately the same time of day (2 P.M.).

In an effort to establish or rule out any correlation between skin reactions to liver extract and pathologic increases of eosinophiles following liver therapy, skin tests were done in most experiments before institution of treatment and again at the end of one month of therapy. For these skin tests 0.05 c.c. of Lilly's liver extract was injected intracutaneously. Experience revealed that the maximum reaction occurred after five minutes. It was arbitrarily decided that any reaction showing pseudopods should be taken as positive. The appearance of wheals per se was not considered representative of a positive reaction, since fourteen of fifteen normal tested individuals developed wheals.

RESULTS

An analysis of the control and anemic cases, with a brief résumé of each, follows:

A. *Controls.*—CASE 1. White male, 32 years old (95023). Diagnosis: delayed union of fractured tibia. The patient was seen in the hospital on five previous admissions when 16 total and differential white blood cell counts were made, and only once was an elevation of

eosinophile count above normal noted, and that to 5 per cent. The initial skin test revealed a wheal approximately 25 mm. in diameter without pseudopods. Lilly's liver extract regimen was then instituted after an initial blood count (see Chart 1). Fifteen days after the beginning of treatment an eosinophilia of 4.25 per cent was noted. A maximum response of 7.75 per cent was attained on the twenty-first day. The skin test at the end of the first month remained essentially unchanged with no pseudopods. After a rest period of ten days the patient received whole cooked liver irregularly for one week, followed by a period of two weeks during which he was given one-half pound of whole cooked liver daily. On the thirtieth day following the institution of the whole cooked liver regimen, a maximum eosinophilia of 7.25 per cent was attained. As shown in Chart 1, a progressive eosinophilia did not occur following either regimen, and with the intramuscular extract eosinophilia did not develop until after liver therapy had been stopped.

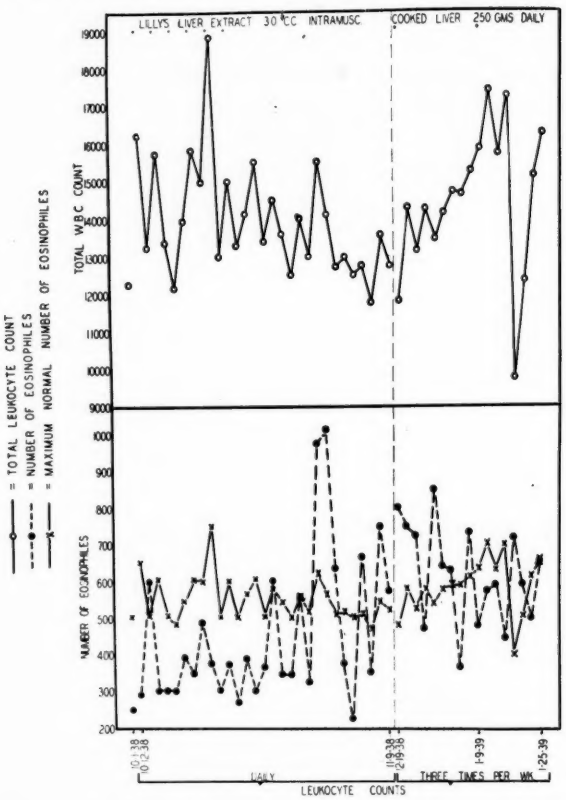

Chart 1.—The curves represent the leucocyte response in a normal individual during treatment with (1) liver extract intramuscularly, 3 c.c. every other day for seven doses; (2) whole cooked liver, one-half pound daily for a period of fourteen days.

CASE 2. White male, aged 22 years (9715). Diagnosis: healed lupus vulgaris (in for plastic repair). The initial skin test revealed no reaction whatsoever. The patient received Lilly's liver extract and later cooked liver, but at no time did the eosinophiles exceed normal limits.

CASE 3. White male, 29 years old (202906). Diagnosis: supracondylar fracture left femur, uncomplicated. The initial skin test revealed a wheal 10 mm. in diameter with no pseudopods. Observation for a period of one month following institution of reticulogen therapy revealed no eosinophilia.

CASE 4. White male, 49 years old (87237). Diagnosis: non-union fracture of left tibia. The initial skin test revealed a wheal 12 mm. in diameter without pseudopods. The patient received reticulogen and subsequently cooked liver. With each of these the eosinophile count reached a maximum of 5 per cent on two occasions. These increases were not considered to be significant.

Analysis of Control Cases.—Of the four control patients only one, Case 1, showed a definite eosinophilia and that was of approximately the same degree following cooked liver and intramuscular liver extract. Skin tests before and after the first regimen were considered to be negative. Skin tests on the remaining three controls were also negative and in none of these did an eosinophilia develop.

B. *Pernicious Anemia.*—CASE 1. White male, 38 years old (107597). The initial skin test revealed a wheal 17 by 17 by 2 mm. with no pseudopods. The patient did not develop eosinophilia during the month's observation when he received 3 c.c. of Lilly's liver extract intramuscularly every five days.

CASE 2. White male, aged 65 years (206698). The initial skin test revealed a wheal 15 by 16 by 2 mm. with two pseudopods about 2 mm. in length. Only one differential leucocyte count was obtained before institution of therapy. The patient developed a slight eosinophilia on the sixth day of parenteral liver extract therapy, but this was irregularly present thereafter with a maximum eosinophilia of 5.75 per cent on the thirteenth day. This level was not considered to be significant. A skin test at the conclusion of the experiment was not obtained.

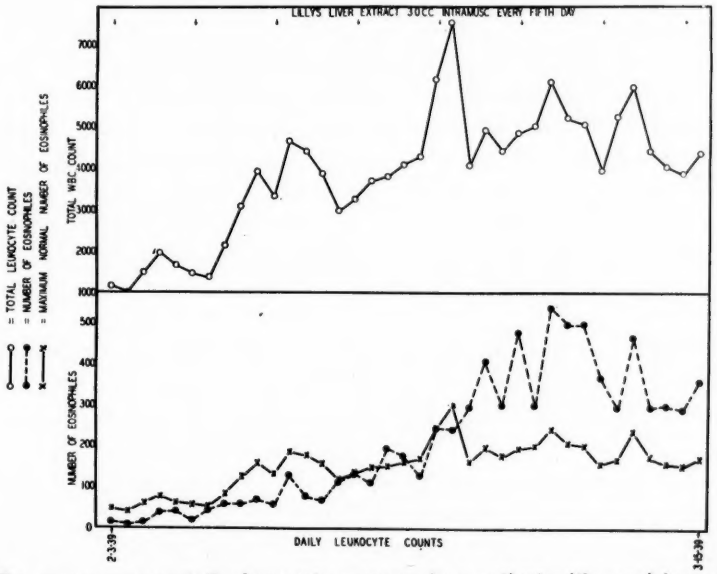

Chart 2.—The curves represent the leucocyte response in a patient with pernicious anemia, previously untreated, while receiving 3 c.c. of liver extract intramuscularly every fifth day.

CASE 3. White female, 50 years old (27360C). The initial skin test showed a wheal 20 by 15 by 2 mm. with three pseudopods, two of which were 10 mm. in length and 2 mm. in width. The third pseudopod was 2 mm. in length. Only one differential count was obtained before parenteral liver therapy was started. Chart 2 depicts the development of the progressive eosinophilia beginning on the eighteenth day and attaining a maximum of 9.75 per cent on the twenty-eighth day. Although liver therapy was continued, the subsequent level of eosinophilia through the thirty-ninth day, the last day of observation, varied between 6.75 and 8.25 per cent.

CASE 4. White female, 60 years old (207666). The initial skin test showed a wheal 14 by 18 mm. with no pseudopods. The patient exceeded the normal percentage of eosinophiles only twice during the month's observation, when she developed a maximum eosinophilia of 5.5 per cent. The skin test at the end of the period of observation revealed a wheal 14 by 13 by 1 mm. with two pseudopods, 2 by 1 mm. and 17 by 2 mm. That a profound eosinophilia would have developed with further observation can only be surmised.

CASE 5. White male, 72 years old (105381). The initial skin test revealed a wheal 14 mm. in diameter with one pseudopod 5 mm. in length. The patient's blood showed a normal eosinophile count throughout the period of observation. A final skin test revealed a wheal 12 by 15 by 0.5 mm. with no pseudopods.

Analysis of Cases With Pernicious Anemia.—Of the five patients with pernicious anemia only one, Case 3, developed a definite eosinophilia. Cases 2 and 3 had positive initial skin tests. Case 1 had a negative initial skin test and developed no eosinophilia. Case 4 had a negative initial skin test and developed no significant eosinophilia but had a positive terminal skin test. Case 5 had a positive initial skin test, developed no eosinophilia, and had a negative terminal skin test.

DISCUSSION

A review of the records of patients with pernicious anemia seen in the State of Wisconsin General Hospital revealed the development of an average maximum eosinophilia varying from 6 to 11.25 per cent in 87 of the 224 persons who had received various forms of liver, and in whom other causes of eosinophilia were eliminated. However, 24, or 53.3 per cent, of the 45 patients who had received no specific treatment and in whom no allergic basis could be discovered showed an eosinophilia, although the average maximum rise was to 5.9 per cent only. No persons in this series received raw liver alone. Of the persons treated with whole cooked liver, 76.9 per cent developed eosinophilia. This incidence of cases developing eosinophilia was higher than that for any group receiving other types of liver therapy. The lowest frequency of eosinophilia coincident with liver treatment was that following oral extract (Lilly's No. 343), being only 16.6 per cent. The instances given in Table I, where only one or two cases received a specific type of treatment, are not considered. Importantly, this review showed that 45.5 per cent of the patients who received intramuscular liver extract developed eosinophilia with an average maximum rise to 11.25 per cent. The degree of eosinophilia in this group surpassed that of any other receiving liver therapy.

It follows, therefore, that eosinophilia may occur in patients with pernicious anemia who have received no specific treatment and in whom there is no obvious allergic or parasitic basis (stool examinations for intestinal parasites were done routinely on all cases of pernicious anemia); that eosinophilia may develop following cooked liver and orally and parenterally administered liver extracts, but apparently more frequently following the former. In no type of therapy, however, is this eosinophilia a regular occurrence.

Although definite deductions could not be made from this small experimental series, the results tend to show that eosinophilia may follow intramuscular liver extract therapy. Further, it is borne out, in agreement with the previously held view,[1, 3] that eosinophilia following liver therapy is not a response peculiar to pernicious anemia. No correlation could be found to exist between skin tests with liver extract and the development of eosinophilia in the peripheral blood. This, of course, does not prove that the eosinophilia is not an allergic phenomenon but rather that skin tests with liver extract, as with many food extracts, may be unreliable. The experimental cases show that eosinophilia may develop after liver therapy has been stopped.

SUMMARY

1. Of the 279 patients with pernicious anemia seen in the State of Wisconsin General Hospital from September, 1925, through September, 1938, 224 received one or more forms of liver therapeutically. Allergic states and parasitic infestation had been excluded in each instance. Of this group of 224 cases, 87 developed eosinophilia at some time or other. However, eosinophilia of lesser degree was found in 24 of 45 cases in whom no evidence of allergy could be found and who had received no form of liver up to the time eosinophilia was discovered. Results of this review are analyzed in Table I.

2. Four control patients, who had no systemic disease and from whom no allergic history could be obtained, were observed for periods of one month following institution of liver therapy. Only one developed an eosinophilia.

3. Five previously untreated patients with pernicious anemia were similarly observed. One person developed definite eosinophilia while receiving parenteral liver extract.

4. An attempt to correlate a local dermal reaction to liver extract and the development of eosinophilia was unsuccessful.

CONCLUSIONS

The feeding of whole liver and the administration of oral and parenteral liver extracts may be followed by the development of eosinophilia in patients with pernicious anemia as revealed by the analysis of the records of such patients seen in the State of Wisconsin General Hospital.

Experimental studies revealed that: (1) Eosinophilia may follow the administration of whole liver and parenteral liver extract in presumably normal individuals; (2) eosinophilia may occur in patients with pernicious anemia following the parenteral administration of liver extract; (3) eosinophilia so induced may persist after cessation of liver therapy.

It has not been demonstrated that the eosinophilia, sometimes found following the administration of liver, is an allergic response nor has this possibility been excluded. Further investigation will be necessary to establish the cause for eosinophilia resulting from liver therapy.

REFERENCES

1. Meulengracht, E., and Holm, S.: Eosinophilia in Liver Diet, Am. J. M. Sc. **179**: 199, 1930.
2. Murphy, W. P.: Anemia in Practice, Philadelphia and London, 1939, W. B. Saunders Co., p. 135.
3. Smith, H. S., and Whitby, L. E. H.: Blood Changes With Liver Diet in Young Convalescents, Lancet **2**: 277, 1928.
4. Watkins, C. H., and Berglund, H.: Analysis of Morphological Blood Changes in Pernicious Anemia Following Administration of Liver, Proc. Soc. Exper. Biol. & Med. **25**: 209, 1927.
5. Whitby, L. E. H., and Britton, C. J. C.: Disorders of the Blood, ed. 2, Philadelphia, 1937, P. Blakiston's Son & Co., p. 92.
6. Whitby, L. E. H.: White Blood Count Changes Resulting From Treatment of Pernicious Anemia With Liver, Lancet **1**: 285, 1928.

PRECIPITIN REACTIONS IN RHEUMATOID ARTHRITIS

J. Graham Bruce, M.D., and Margaret Caswell, A.B.
Springfield, Mass.

SINCE the clinical course and pathologic findings of rheumatoid arthritis differ from osteo-arthritis, it is reasonable to presume that they also have separate etiologic factors. In the etiology of rheumatoid arthritis, the contributing factors are many, such as environment, heredity, avitaminosis, faulty metabolism, infection, and psychic trauma. Of all these factors, infection is believed to play a prominent role, and the *Streptococcus hemolyticus* is implicated in some way as suggested by the agglutination reaction.[1] The infection hypothesis is bolstered also by the fact that rheumatoid cases show an elevated sedimentation rate, a rise in serum globulin and a lowering of serum albumin, an elevated filament-nonfilament count, and a positive agglutination and precipitin reaction to *Streptococcus hemolyticus.*[2]

In this study we are presenting a series of cases of arthritis in which streptococcus precipitin reactions were done. Comparable agglutination reactions have been done by numerous investigators. Nicholls and Stainsby[3] in 1931, using a "typical strain" of *Streptococcus hemolyticus,* obtained a positive agglutination in "practically all cases." In the following year Clawson and Weatherby,[4] and Dawson, Olmstead, and Boots,[5] found that 59 and 67 per cent, respectively, of rheumatoid types presented a positive agglutination. Wainwright[6] in 1934 observed that the blood sera of 90 per cent of 87 cases of rheumatoid arthritis were positive. He used both living and heat-killed antigens. Nearly all these men used a "typical strain" of *Streptococcus hemolyticus.*

METHOD

Using the hydrochloric acid extract as antigen, Neil and Hartung[7] in 1937 showed the precipitin reaction to be comparable to the agglutination reaction, in which live bacteria were used. We, therefore, used the hydrochloric acid extract as antigen because of the difficulty in keeping a growth of streptococcus alive. The method of obtaining the extract from a culture of *Streptococcus hemolyticus,* using the Lancefield method, Porge modification, is as follows: a liter of plain broth having pH 7.6 is inoculated with 6 c.c. of fresh growing broth culture of *Streptococcus hemolyticus* and incubated at 37° C. for eighteen hours. The broth culture is then centrifuged and the precipitate is suspended in 0.85 per cent sodium chloride. Normal hydrochloric acid is added to give a resulting concentration of twentieth-normal. This acidified suspension (volume approximately 15 c.c.) is immersed in boiling water for fifteen minutes, then cooled to room temperature and centrifuged for thirty minutes. The resulting

Received for publication, November 15, 1939.

supernatant is neutralized with sodium hydroxide, and the precipitate is discarded after centrifuging. The crystal-clear, slightly yellowish supernatant is the crude antigen and is ready for use.

The type-specific fraction designated as "M" may be obtained from this crude antigen by a series of extractions, using ethyl alcohol and sodium acetate. However, since Neil and Hartung[7] in their study demonstrated that the crude antigen gave as many positive precipitin tests as the refined alcoholic extract containing the type-specific "M" fraction, and less than 0.1 per cent carbohydrate, only the crude antigen was used in this series. Three different antigens were used in the course of these precipitin reactions. The organism used in all three antigen preparations was a *Streptococcus hemolyticus*, beta type, freshly isolated from pathogenic sources.

TABLE I

PRECIPITIN REACTION IN PATIENTS WITH RHEUMATOID AND MIXED ARTHRITIS

CASE NO.	PATIENT	SEX	AGE	DURATION OF DISEASE	PRECIPITIN	TYPE	REMARKS
1	F. M.	F	68	15 years	OOO	Mixed	
2	Miss C. T.	F	45	4 years	XOO	Rheumatoid	
3	Mrs. B.	F	49	16 years	XXX	Rheumatoid	
4	C. C.	F	56	9 years	OOO	Rheumatoid	Arthritis practically burned out
5	A. Boe	F	64	8 years	OOO	Mixed	
6	M. Bas.	F	23	9 months	OOO	Rheumatoid	
7	L. F.	F	56	9 months	XXO	Mixed	
8	C. F.	F	46	3½ years	XXX	Rheumatoid	
9	M. M.	F	43	3 years	XXX	Rheumatoid	
10	Miss P.	F	38	8 years	XXX	Rheumatoid	
11	Miss Tim	F	70	14 years	OOO	Rheumatoid	
12	Mrs. P.	F	64	18 years	X--	Rheumatoid	
13	Mrs. M. B.	F	47	2 years	OOO	Mixed	
14	M. R.	F	66	2 years	XXO	Mixed	
15	M. Bo.	F	52	6 months	OOO	Mixed	
16	A. Bas.	F	26	3 years	XXO	Rheumatoid	
17	Mrs. G. St.	F	53	3 years	XXX	Rheumatoid	
18	Mrs. J. Ha.	F	37	7 months	OOO	Rheumatoid	
19	T. L.	M	33	5 years	XXO	Rheumatoid	
20	S. W.	F	46	1 year	OOO	Rheumatoid	
21	Mr. V. D.	M	36	5 months	XOO	Rheumatoid	
22	E. Y.	F	62	8 years	XOO	Rheumatoid	
23	F. P.	F	44	24 years	XOO	Rheumatoid	
24	L. T.	M	62	3 years	XXX	Rheumatoid	
25	W. R. R.	M	67	3 weeks	XX-	Rheumatoid	
26	R. N.	F	48	3 weeks	XOO	Rheumatoid	
27	E. B.	F	60	25 years	XXX	Mixed	
28	I. MacP.	F	47	4 weeks	XXO	Mixed	
29	K. H.	F	64	9 years	XXO	Rheumatoid	
30	D. J.	M	35	2 months	XOO	Rheumatoid	
31	M. F.	M	52	1½ years	XOO	Rheumatoid	
32	E. Ois	F	28	4 years	OOO	Rheumatoid	

RESULTS

In 32 cases of the rheumatoid or mixed type there were 22, or 68.7 per cent, whose sera gave positive precipitin reactions. These results compare favorably with those of other workers, mentioned previously in this report. The 13 cases of osteo-arthritis, with but a few exceptions, gave negative results. The normal controls were also all negative.

TABLE II

PRECIPITIN REACTIONS IN PATIENTS WITH OSTEO-ARTHRITIS

CASE NO.	PATIENT	SEX	AGE	DURATION OF DISEASE	PRECIPITIN	REMARKS
1	Mrs. E. A.	F	56	2 years	OOO	
2	Mrs. C. D.	F	66	8 months	OOO	
3	A. C.	F	52	6 months	OOO	Agglutination, negative 1-80
4	L. H.	F	56	8 years	XXX	
5	Mrs. M. K.	F	53	2 months	XOO	Also had varicose ulcer
6	E. K.	F	38	4 years	OOO	
7	J. Le.	M	62	2 months	OOO	
8	J. Li.	M	49	8 years	OOO	
9	Miss T.	F	73	10 years	OOO	
10	Mrs. P.	F	61	5 years	XXX	
11	L. C.	M	60	6 months	OOO	
12	S. H.	M	50	10 days	OOO	
13	F. P.	F	49	10 years	OOO	
14	A. Sig.	F	80	8 years	OOO	
15	M. A.	F	53	2 months	XOO	

TABLE III

PRECIPITIN REACTIONS IN NORMAL CONTROLS

CASE NO.	PATIENT	SEX	AGE	PRECIPITIN
1	M. C.	F	23	OOO
2	D. H.	F	25	OOO
3	N. A.	F	24	OOO
4	J. G. B.	M	34	OOO
5	W. L.	M	32	OOO
6	J. Z.	M	23	OOO
7	S.	M	25	OOO
8	V. S.	F	24	OOO
9	L. S.	M	28	OOO
10	K. K.	M	25	OOO

Thus, it would seem that in the light of our present knowledge concerning arthritis, the *Streptococcus hemolyticus* is in some way implicated. Arthritis has been produced experimentally in rabbits by injecting small amounts (0.1 to 2 c.c.) of a broth culture of *Streptococcus hemolyticus* intravenously. The sedimentation rates, as well as the streptococcus agglutinins, were increased in these animals, whether they developed arthritis or not.[8] These observations tend to make one suspect the streptococcus as an etiologic factor, except that the pathology produced in the joints clears up in a short time.

However, it is more likely that the agglutination test is nonspecific. The reaction is highly characteristic for atrophic arthritis, and Hench says "does not bar the conclusion that the agglutinins present in these sera are the result of rheumatoid disease."[9] The Wassermann reaction is nonspecific in the sense that it is not a specific test for the presence of the spirochete. By the same token the agglutination and the precipitin reactions may be specific for atrophic arthritis, but without etiologic significance.

Other workers[10] have shown that the agglutination titer may change from month to month on the same patient without any change in the sedimentation rate or clinical course of the disease. These men feel that the sedimentation rate is better as a diagnostic test than the agglutination reaction.

The ubiquitous streptococcus can be implicated; this is about all that can be said. Most authors agree with this postulate. More work to uncover the exact etiology of rheumatoid arthritis will have to be done before the exact role which the streptococcus plays can be determined. For the present, we may only say that the precipitins, as well as the agglutinins, in the sera of patients with rheumatoid arthritis are indirect evidence that infection plays a role in the etiology of this disease.

CONCLUSIONS

1. In a series of 32 cases of rheumatoid arthritis, the sera of 22 showed precipitins to the *Streptococcus hemolyticus* in high titer.

2. Four out of 15 cases of osteo-arthritis had a positive precipitin reaction, and none of the normal controls were positive.

3. The precipitin and agglutination reactions to the *Streptococcus hemolyticus* are indirect evidence that the streptococcus plays a part in rheumatoid arthritis.

REFERENCES

1. Boots, Ralph H.: The Essential Features in the Differential Diagnosis of Rheumatoid and Osteoarthritis, J. LAB. & CLIN. MED. **22**: 18, 1939.
2. Hartung, E. F., Davis, J. Z., Steinbrocher, O., and Straub, M. E.: The Blood in Arthritis, J. A. M. A. **106**: 1448, 1936.
3. Nicholls, E. E., and Stainsby, W. J.: Streptococcal Agglutinins in Chronic Infectious Arthritis, J. Clin. Investigation **10**: 323, 1931.
4. Clawson, B. J., Wetherby, M., Hilbert, E., and Hilleboe, H. E.: Streptococcic Agglutination in Chronic Arthritis and Acute Rheumatic Fever, Am. J. Med. Sc. **184**: 768, 1932.
5. Dawson, M. H., Olmstead, M., and Boots, R. H.: Agglutination Reactions in Rheumatoid Arthritis, J. Immunol. **23**: 187, 1932.
6. Wainwright, Charles W.: Treatment of Chronic Atrophic (Rheumatoid) Arthritis With Streptococcus Vaccine on the Basis of Skin Sensitivity, Third Conference on Rheumatic Disease, J. A. M. A. **103**: 1733, 1934.
7. Neil, Margaret Straub, and Hartung, E.: Precipitins for Streptococcus hemolyticus in Rheumatoid Arthritis Serums, J. LAB. & CLIN. MED. **22**: 881, 1937.
8. Angevine, D. Murray, Cecil, Russell L., and Rothbard, Sidney: Immunologic and Bacteriologic Observations in Experimental Streptococcic Arthritis, J. A. M. A. **111**: 2041, 1938.
9. Hench, P. S., Bauer, W., Dawson, H., Hall, F., Holbrook, W. P., and Key, J. A.: The Problem of Rheumatism and Arthritis—Review of American and English Literature for 1937, Ann. Int. Med. **12**: 1005, 1939.
10. Short, C. L., Dienes, L., and Bauer, W.: Rheumatoid Arthritis—A Comparative Evaluation of the Commonly Employed Diagnostic Tests, J. A. M. A. **108**: 2087, 1937.

LEUCOCYTE CHANGES IN ACUTE PERITONEAL IRRITATION*

ROBERT E. KAUFMAN, M.D., AND FREDERICK VOM SAAL, M.D.
NEW YORK, N. Y.

SINCE the introduction of the Schilling count[1] into clinical hematology, there has been much interest in the so-called "shift to the left." This is a term used to indicate an increased percentage of immature polymorphonuclear leucocytes in the differential count. The adult polymorphonuclear cells have two or more distinct lobes, connected by a very fine, threadlike filament. Any polymorphonuclear cell without this definite filament is not adult, even if there are two or more lobes. There are many different terms used to contrast these two types of cells, such as mature and immature; adult and young; segmented and nonsegmented; segmenters and band forms; filamented and nonfilamented; segmented and juvenile plus stab or staff cells. It is now customary to group together the juvenile and stab cells of Schilling's classification, and to call all polymorphonuclear cells either mature or immature.[2, 3]

There is considerable difference of opinion concerning the normal percentage of immature polymorphonuclear leucocytes in the capillary blood. Some[1, 4, 5] feel that normally there are only 3 to 6 per cent of these cells compared to the total number of white cells; and that anything above 5 per cent should be considered a shift to the left. Most of the more recent observers, however, follow the teaching of other authorities[2, 6-8] and find a normal of 5 to 10 per cent, with the upper limit at 10 to 12 per cent. We consider 4 to 12 per cent normal; 13 to 20 per cent a slight shift; 21 to 39 per cent a moderate shift; and 40 per cent and over a marked shift.

The percentage of immature polymorphonuclear leucocytes has considerable diagnostic and prognostic value.[1, 3, 9, 10] It is often assumed that a shift to the left indicates only the presence of infection. However, a shift may also be found in rupture of a viscus, thrombosis (coronary and elsewhere), hemorrhage, pregnancy, malignant neoplasms, acute ileus,[11] bone marrow irritation, such as that due to metastatic infiltration, and following various types of acute peritoneal irritation.

Since 1933, when the Schilling differential count was introduced as a routine in this hospital, we have been impressed with the constancy of the left shift, often extremely marked, in cases of acute peritoneal irritation. This observation has at times aided considerably in diagnosis. We, therefore, think it of interest to report a series of 132 differential counts in a group of 104 patients with various kinds of acute peritoneal irritation, proved by operation or autopsy.

*From the Lenox Hill Hospital, New York.
Received for publication, November 16, 1939.

Several sources of error in our data must be recognized. The counts were made by 58 interns; hence the human error must be considered. In addition, there is a moderate statistical error inherent in counting only 100 to 200 cells.[12, 13] As a result of these factors, the figures represent the usual conditions of medical practice in most hospitals.

"PRIMARY" PERITONITIS

The group of 9 cases of "primary" peritonitis is most illuminating in this connection. By primary peritonitis we mean "those cases which suddenly develop peritonitis unassociated with evidence of a pre-existing abdominal inflammation."[14] Of the 9 counts (Table I), all except one (32 per cent) show a marked increase in immature polymorphonuclear leucocytes (44 to 74 per cent). The patient with only 5,750 white blood cells had the highest percentage of immature cells (74 per cent).

TABLE I

"PRIMARY" PERITONITIS

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
1	5,750	92	74	10 hours
2	18,900	64	32	24 hours
3	21,000	98	44	11 hours
4	23,150	89	50	18 hours
5	24,750	82	47	72 hours
6	24,800	95	58	4 hours
7	29,600	88	72	5 days
8	34,000	99	47	4 days
9	35,000	98	44	15 hours
Average		89.4	52.0	

PERFORATED PEPTIC ULCER

Table II shows the counts of 24 cases of perforated peptic ulcer. It will be noted that the shift is not so constant in this group of cases as in the preceding. On the whole, however, there is a definite trend for a rather marked shift. One example will suffice to show how rapidly the immature cells may be poured into the peripheral blood after a perforation of an abdominal viscus.

A 38-year-old man was admitted to the hospital for a partial pyloric obstruction from a cicatrized ulcer. There was no suggestion of a perforation; his temperature and pulse were normal; his differential count showed 86 per cent polymorphonuclear cells, of which only 8 were immature. Twenty-four hours after admission the patient complained of sudden onset of terrific epigastric pain, and the diagnosis of perforated peptic ulcer was made clinically. Fifteen minutes after the onset of pain one of us did a differential count and found 92 per cent polymorphonuclear cells, of which 24 were immature. One and a half hours after the perforation, just before operation, the count was repeated by the same observer, and there were now 97 per cent polymorphonuclear cells, of which 39 were immature. Operation confirmed the diagnosis.

PERFORATION OR GANGRENE OF SMALL INTESTINE

We have recorded in Table III the results of 17 counts done on 6 patients with perforation or gangrene of the small intestine. These persons, including those with gangrene in whom a definite perforation could not be found, all had purulent peritoneal fluid containing organisms. The large number of counts

TABLE II

PERFORATED PEPTIC ULCER

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
10	9,000	67	18	3 hours
11	9,350	75	31	1 hour
12	9,960	91	46	8 hours
13	12,100	95	22	10 hours
14	13,000	90	30	1 hour
15	13,650	90	22	18 hours
16	13,750	98	39	3 hours
17	14,500	85	46	24 hours
18	14,750	90	9	6 hours
19	15,000	78	28	4 hours
20	15,050	88	47	2 hours
21	15,200	90	20	10 hours
22	15,650	88	47	5 hours
23	15,800	84	26	17 hours
24	15,800	80	32	4 hours
25	16,500	94	36	6 days
26	16,550	94	81	16 hours
27	18,600	66	13	3 hours
28	18,700	87	18	6 hours
29	19,150	98	18	5 hours
30	21,800	92	36	4 hours
31	22,500	78	18	10 hours
32	26,400	88	32	7 hours
33	Not done	92	24	¼ hour
33	Not done	97	39	1½ hours
Average		86.5	31.1	

TABLE III

PERFORATION OR GANGRENE OF SMALL INTESTINE

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
34	3,650	64	63	?
34	4,700	65	48	6 hours
35	5,600	90	40	3½ days
36	7,000	79	40	4 days
37	7,050	59	37	5 days
35	7,200	74	27	54 hours
38	7,300	79	69	7 days
36	10,000	48	30	5 days
35	13,200	84	29	30 hours
35	15,000	90	10	6 hours
39	17,200	84	24	15 hours
39	17,800	86	18	4 hours
35	18,000	96	24	18 hours
37	19,500	92	24	12 hours
39	24,000	90	28	10 hours
37	28,500	80	22	24 hours
36	Not done	61	44	6 days
Average		77.7	34.0	

per person indicates the difficulty of arriving at a diagnosis in this type of case, and the consequent delay in operating. It is important to note that in the cases with a low total white blood cell count the shift to the left is usually most marked. The practical significance of this observation is evident, since it is in those cases with a low total white blood cell count that the surgeon tends to delay unnecessarily. An example will illustrate this point.

A 39-year-old German bartender was admitted to the hospital with a history of four days of dull, intermittent midabdominal pain, anorexia, nausea and frequent vomiting. For the past ten years he had had a right inguinal hernia, which had never given him any trouble.

On examination he was found to have marked congestion of the tonsils and pharynx, a few small axillary and epitrochlear nodes, and a right indirect inguinal hernia with a relaxed external ring, admitting one finger. His temperature was 101.2° F. and his pulse was 100 on admission, but both came down to normal within twelve hours and remained normal. The initial blood count showed 7,000 white blood cells; polymorphonuclear cells 79 per cent, of which 40 were immature; lymphocytes 21 per cent. The next day the total count was 10,000; polymorphonuclear cells 48 per cent, of which 30 were immature; lymphocytes 42 per cent; monocytes 6 per cent; eosinophiles 4 per cent. The following day the total white blood cell count was not determined, but the differential count showed polymorphonuclear cells 57 per cent, of which 40 were immature; myelocytes 4 per cent; lymphocytes 24 per cent; monocytes 11 per cent; eosinophiles 4 per cent. During the first four days in the hospital he had no symptoms, but on the fifth day he developed pain in the right inguinal region. The external ring was found filled with intestines. Shortly thereafter the patient reduced the hernia himself. Operation was nevertheless advised, but was not considered urgent. The patient insisted on going home, for he felt well and wished to return to work. The next afternoon he returned in shock, after having vomited all night. He died two hours after readmission. Autopsy revealed two areas of gangrene, 6 cm. apart, in the distal ileum, one recent and the other old.

PERFORATED APPENDIX

The cases of perforated appendix, either without abscess or with an abscess of less than five days' duration, are listed in Table IV. In this group also there is a tendency for those cases with a low total white blood cell count to have a more marked shift than those with a higher count.

To show how rapidly and dramatically the blood picture can change, we wish to mention the case of a 2-year-old female child who was admitted to the hospital with symptoms and signs suggesting an acute abdominal inflammation. The initial count, made forty-eight hours after the onset of symptoms, was as follows: white blood cells 7,800; polymorphonuclear cells 62 per cent, of which but 22 were immature; lymphocytes 38 per cent. This relatively normal count was in part responsible for a short delay in asking the surgeons to see the child. Five hours later the count was white blood cells 4,000; polymorphonuclear cells 80 per cent, of which 70 were immature; lymphocytes 20 per cent. Operation was performed immediately after this count, and a perforated appendix was found.

PERFORATION OF SIGMOID DIVERTICULI

In Table V we have recorded the results of 6 counts in 4 cases of perforation of sigmoid diverticuli. All the counts, with one exception, show a marked shift to the left. Here again the low total counts were accompanied by a high immature count, even when the polymorphonuclear cells were not markedly increased. The one case in which no significant shift was recorded had a total white blood cell count of 33,800 and a polymorphonuclear cell count of 93 per cent.

PERFORATED GALL BLADDER

It is well recognized by clinicians that the severity of most cases of acute cholecystitis is extremely difficult to evaluate, and that an empyema or perforation of the gall bladder may be present with very slight symptoms and signs or changes in the blood picture.[15-17] In our small series of 6 proved cases of perforated gall bladder only 1 out of a total of 9 counts showed a greater percentage of immature cells than 32 (Table VI). It is unfortunate, but apparently true, that the degree of shift cannot be counted on as an aid in diagnosing the severity of a cholecystitis.

TABLE IV

PERFORATED APPENDIX

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
40	4,000	80	70	53 hours
41	5,400	65	34	72 hours
42	6,750	86	41	60 hours
43	7,150	94	74	48 hours
40	7,800	62	22	48 hours
44	9,100	86	40	72 hours
45	10,400	72	11	54 hours
46	10,600	59	25	4 days
47	11,500	90	52	5 days
48	11,850	91	29	5 days
49	13,200	86	35	24 hours
50	13,400	95	10	72 hours
51	13,700	85	33	36 hours
52	14,500	85	22	25 hours
53	14,700	85	48	48 hours
54	15,000	88	30	72 hours
55	16,200	95	40	48 hours
56	16,800	80	20	4 days
57	17,200	86	16	48 hours
58	17,700	93	10	4 days
59	18,400	90	32	72 hours
60	18,600	96	48	36 hours
61	18,800	88	26	24 hours
62	19,200	88	25	36 hours
63	19,900	76	15	48 hours
64	20,000	91	20	25 hours
65	20,450	89	28	4 days
66	21,400	90	25	4 days
67	24,200	90	9	14 hours
68	24,500	96	20	48 hours
69	24,800	90	29	6 hours
70	26,800	83	45	4 hours
71	27,100	92	68	28 hours
72	27,500	96	40	21 hours
73	29,000	85	28	?
74	30,700	88	19	23 hours
75	30,700	93	32	50 hours
Average		86.0	31.6	

TABLE V

PERFORATED SIGMOID DIVERTICULI

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
76	7,900	84	42	18 hours
77	9,090	76	50	52 hours
77	9,500	72	42	18 hours
77	13,950	86	57	40 hours
78	24,150	92	74	14 days
79	33,800	93	16	72 hours
Average		83.8	46.8	

HEMORRHAGIC PANCREATITIS

The cases of acute hemorrhagic pancreatitis in our series (Table VI) are too few to be discussed in detail. There is a tendency for an extremely high polymorphonuclear cell percentage and for a marked shift to the left, although the latter is not consistently noted. In typical instances of this disease, the aid which may be had from the blood count is unnecessary, as the clinical picture of

a surgical abdominal emergency is so striking. If one must attempt to distinguish between hemorrhagic pancreatitis and coronary occlusion, as occasionally happens, it would appear that a very high polymorphonuclear count and a marked shift, especially if they occur within a few hours after the onset of symptoms, favor the former diagnosis.

TABLE VI

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
Perforated Gall Bladder				
80	4,450	77	3	5 days
81	10,500	80	32	4½ days
82	13,500	93	68	32 hours
83	15,800	86	12	2 hours
84	19,500	92	10	?
83	20,400	84	12	63 hours
83	22,500	96	30	45 hours
83	23,000	88	24	19 hours
85	38,000	96	24	21 hours
Average		88.0	23.9	
Hemorrhagic Pancreatitis				
86	13,600	89	42	16 hours
86	14,500	90	56	42 hours
87	15,900	90	46	?
88	21,000	98	29	72 hours
88	26,150	96	21	4 days
Average		92.6	38.8	

TABLE VII

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
Ruptured Ectopic Pregnancy				
89	5,650	76	38	42 hours
89	9,850	86	65	6 hours
89	11,300	76	17	20 hours
90	15,000	88	34	6 days
89	25,000	84	26	13 days
91	29,000	85	32	50 hours
Average		82.5	35.3	
Ruptured Graafian Follicle and Corpus Luteum Cyst				
92	7,500	78	6	15 hours
92	8,300	84	13	60 hours
93	8,400	80	15	25 hours
92	8,600	84	32	72 hours
94	9,800	87	25	1 hour
95	11,200	83	12	4 hours
96	13,400	71	18	4 days
97	14,100	84	52	9 hours
98	19,300	86	22	48 hours
Average		81.9	21.7	

RUPTURED ECTOPIC PREGNANCY, GRAAFIAN FOLLICLE, CORPUS LUTEUM CYST

A sudden and profuse hemorrhage into the peritoneal cavity may give rise to a marked shift to the left, similar to an infectious process; but usually if the hemorrhage is not large or sudden, the shift is not noteworthy. Three cases of ruptured ectopic gestation and seven cases of rupture of a Graafian follicle or

of a corpus luteum cyst are recorded in Table VII. The presence of a marked shift in a patient suspected of having had a rupture of an ectopic pregnancy is of considerable aid in deciding on an immediate operation. On the other hand, it is reassuring to note the low immature counts of the cases of rupture of a Graafian follicle or corpus luteum cyst, as these conditions do not require operation.

One patient who was known to be about two months pregnant had a white blood cell count of 9,850, with 86 per cent polymorphonuclear cells and 65 immature cells six hours after the onset of vague abdominal complaints. As the symptoms and signs were mild and as they rapidly subsided, she was allowed to go home. She returned thirteen days later, was immediately operated upon for a ruptured ectopic pregnancy, and died within a few days from uremia. If the extremely marked shift found on admission, and undoubtedly due to a sudden profuse intra-abdominal hemorrhage, had received due weight, this patient's life might well have been saved.

TABLE VIII

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT	DIAGNOSIS
99	4,700	51	42	6 days	Volvulus
99	6,800	63	59	6½ days	Volvulus
100	8,600	95	83	6 hours	Site unknown
101	12,000	96	46	10 hours	Site unknown
102	14,300	87	41	12 hours	Perforated Fallopian tube
99	15,150	82	26	2 days	Volvulus
99	16,100	71	56	5 days	Volvulus
103	16,500	92	15	52 hours	Periappendicitis
104	20,600	91	42	72 hours	Periappendicitis
Average		80.9	44.2		

MISCELLANEOUS CASES OF ACUTE PERITONEAL IRRITATION

Various types of acute peritoneal irritation are gathered together in Table VIII, and it will be seen that the one thing these cases have in common is a sudden peritoneal insult. Most of them show the typical marked shift, which, when present with abdominal symptoms, is very important diagnostically. One case will well illustrate the value of understanding the significance of a high immature count in an acute abdominal condition.

A 61-year-old female had been on the medical ward for ten weeks with hypertensive cardiovascular renal disease. She had been decompensated on admission, but was doing very well when she was awakened one morning by rather severe generalized abdominal pain. She vomited several times, perspired profusely, and had cold extremities. Examination soon after the onset of symptoms revealed diffuse abdominal tenderness, especially in the left upper quadrant. Her temperature was 98.0° F.; her pulse was 70; and her respirations were 24 per minute. We were under the impression that we were dealing with an acute abdominal condition of some sort. A blood count, performed shortly after the examination or five hours after the onset of the pain, was as follows: white blood cells 8,600; polymorphonuclear cells 95 per cent, of which 83 were immature. Because of the extreme shift to the left, a diagnosis of acute peritonitis seemed almost certain. For this reason, an abdominal tap was immediately performed and 1 c.c. of turbid, straw-colored fluid with numerous flecks of fibrin was removed. A smear showed a variety of gram-positive and gram-negative bacilli and cocci, later substantiated by culture. The organisms obviously entered the abdominal cavity from the intestinal tract, probably as the result of a leak through the chronically distended gut or following a mesenteric thrombosis. Because of the patient's poor cardiovascular condition, operation was deemed inadvisable, and strangely enough, the patient made an uneventful recovery in a few days and subsequently went home.

DISCUSSION

We wish to emphasize that in the presence of suspected abdominal pathology, a high percentage (over 35 to 40 per cent) of immature polymorphonuclear leucocytes in the peripheral blood suggests acute peritoneal inflammation, almost always requiring immediate surgical intervention. In Table IX the immature cell percentages of all the counts are averaged according to the number of hours elapsing from the onset of symptoms to the time the count was done. This grouping brings out the fact that in this series the average immature count is almost identical in the group in which the counts were done shortly (less than ten hours) after the onset of symptoms and in the group in which many hours or even days elapsed. It is, therefore, apparent that the shift does not depend so much on infection as on sudden peritoneal shock or irritation. Even a very few hours after the perforation of a gastric ulcer, for example, there is usually noted a considerable shift. This is evidently not dependent on an infection, but is probably due to an inflammation of a chemical nature.

TABLE IX

ONSET OF SYMPTOMS TO TIME OF COUNT	AVERAGE PER CENT IMMATURE POLYS.
Under 10 hours	34.9
11-30 hours	33.3
Over 30 hours	34.3

TABLE X

TOTAL W.B.C.	AVERAGE PER CENT IMMATURE POLYS.
Under 10,000	41.2
10,000-20,000	30.2
20,000-30,000	34.7
Over 30,000	30.3

While one should consider the blood count as a whole, our experience with this series of patients makes us feel strongly—as do many others[1, 3, 9]—that the degree of shift is the most important aspect of the hematologic picture in acute abdominal conditions, with the exception of gall bladder pathology. It must be realized, however, that a slight or absent shift does not by any means rule out shock or severe intra-abdominal disease. It is of the utmost importance to recognize the significance of a high percentage of immature polymorphonuclear leucocytes in the presence of a low total white blood cell count. It will be seen from Table X that the greatest shift on the average was in those cases in which the total white blood cell count was "normal," i.e., under 10,000. It is often in the patients with normal total white blood cell counts that delay in operation seems safe to the surgeon, whereas less attention to the total and more attention to the degree of shift would aid considerably in diagnosis and prognosis.

CONCLUSIONS

1. A marked shift to the left in the differential blood smear is frequently present in all types of acute peritoneal irritation, even without infection.

2. The absence of a marked shift does not eliminate the possibility of acute peritoneal irritation.

3. In the presence of a low total white blood cell count, a definite shift is of especial importance in diagnosis and prognosis.

4. Repeated differential smears, properly interpreted, aid materially in deciding when and whether to operate for a suspected surgical abdominal emergency.

We wish to express our sincere thanks and appreciation to Dr. Henry Henstell for considerable aid in the preparation of the manuscript. Also to Miss Victoria Braun and her staff of the record room for their cheerful cooperation.

REFERENCES

1. Schilling, V.: The Blood Picture and Its Clinical Significance, St. Louis, 1929, The C. V. Mosby Co.
2. Farley, D. L., St. Clair, H., and Reisinger, J. A.: The Normal Filament and Nonfilament Polymorphonuclear Neutrophil Count: Its Practical Value as a Diagnostic Aid, Am. J. M. Sc. **180**: 336, 1930.
3. Haden, R. L.: The Interpretation of Qualitative Changes in Neutrophilic Leukocytes, Cleveland Clin. Quart. **5**: 184, 1938.
4. Osgood, E. E.: Normal Hematologic Standards, Arch. Int. Med. **56**: 849, 1935.
5. Crocker, W. J., and Valentine, E. H.: Hemography in Diagnosis, Prognosis and Treatment Based on 6,000 Schilling Hemograms, J. LAB. & CLIN. MED. **20**: 172, 1934.
6. Cooke, W. E., and Ponder, E.: The Polynuclear Count, Philadelphia, 1927, J. B. Lippincott Co.
7. Reznikoff, P.: Immature White Blood Cell Counts in Infectious Diseases, J. A. M. A. **93**: 963, 1929.
8. Needles, R. J.: A Neutrophilic Graph, J. LAB. & CLIN. MED. **17**: 962, 1932.
9. Schilling, V.: Clinical Interest in the Enumeration of the Staff Cells, Rev. belge sc. méd. **10**: 264, 1938.
10. Klöckner, G.: The Blood Picture as an Aid in Diagnosis and Indication for Operation of Atypical and Typical Cases of Peritonitis, Med. Welt. **9**: 1622, 1935.
11. Demidova, P. N.: Blood Picture in Acute Ileus, Vestnik khir. **56**: 356, 1938 (abstracted in J. A. M. A. **112**: 1775, 1939).
12. Mainland, D., Du Bilier, B., and Stewart, C. B.: The Accuracy of Differential Blood Counts, Canad. M. A. J. **33**: 667, 1935.
13. Goldner, F. M., and Mann, W. N.: The Statistical Error of the Differential White Count, Guy's Hosp. Rep. **88**: 54, 1938.
14. Duncan, J. H.: Primary Peritonitis: Pneumococcic and Streptococcic, Canad. M. A. J. **24**: 778, 1931.
15. Douglas, J.: Acute Inflammation of the Gall-Bladder and Biliary Ducts, New York State J. Med. **37**: 1119, 1937.
16. Wesson, H. R., and Montgomery, T. R.: Acute Cholecystitis: Report of Eighty-seven Cases, Proc. Staff Meet., Mayo Clin. **12**: 500, 1937.
17. Glenn, F.: Acute Cholecystitis, Surg., Gynec. & Obst. **69**: 431, 1939.

THE EFFECT OF BELLADONNA ON THE APPETITE OF PATIENTS WITH OBESITY AND WITH OTHER DISEASES*

JAMES A. GREENE, M.D., IOWA CITY, IOWA

IT WAS noted in a previous report[1] that relative inactivity was an important factor in the production of excess body weight in a majority of obese patients. These findings indicate that the appetite was not reduced in proportion to the activity. That there is a disturbance in control of appetite in such patients is supported by the additional fact that many patients state that hunger prevents them from adhering to a low caloric diet. Our interest in the control of hunger by medication was aroused several years ago when a patient stated repeatedly that she was able to follow a low caloric diet only so long as she took a medication which contained belladonna and bromide. This observation caused us to prescribe tincture of belladonna alone, or in combination with bromide or with phenobarbital, to other patients without their knowledge of the purpose.

TABLE I

DIAGNOSIS	TINCTURE BELLADONNA AND BROMIDES		TINCTURE BELLADONNA		TINCTURE BELLADONNA AND PHENOBARBITAL		TOTAL
	APPETITE		APPETITE		APPETITE		
	DECREASED	NOT AFFECTED	DECREASED	NOT AFFECTED	DECREASED	NOT AFFECTED	
Obesity	24	3	3		7	2	39
Diabetes and obesity			1		2		3
Diabetes			1		1		2
Chronic pyelonephritis			1				1
Total	24	3	6	0	10	2	45

It is to be noted from Table I that the appetite was diminished in 40 of 45 patients following the administration of such medication. These results, we believe, are higher than would be obtained in a larger series of cases. That the medication was an important factor in reduction of appetite is shown by the fact that ten patients who omitted the medication of their own accord resumed it because they noted that hunger returned. The duration of the effect has varied from as short as three weeks to as long as two years. The duration of the effect in most patients appears to continue indefinitely as long as the medication is continued. One patient with diabetes and moderate obesity was of particular interest. She had never been able to control the diabetes because

*From the Department of Internal Medicine, State University of Iowa, College of Medicine, Iowa City.

Received for publication, November 17, 1939.

hunger caused her to eat between meals. The administration of tincture of belladonna, 10 drops three times daily, before meals, eliminated this hunger and permitted her to control the diabetes.

Another patient presented an unusual case. She was forced to limit her activity because of chronic pyelonephritis and hypertension. She began to gain weight following this diminished activity. The administration of tincture of belladonna decreased her food intake sufficiently to stop the gain in body weight. Omission of the drug at a later date was followed by gain in body weight and a constant body weight again followed the administration of the drug. The prescribed diet was not altered during these periods of observations, and she voluntarily stated that her appetite was diminished during the periods of medication.

The cause of the increase in appetite in patients with obesity is not known. Harrington[2] and Newburgh[3] concluded from their studies that many patients ingested food at frequent intervals because of nervousness or of habit. Our observations support this contention in certain patients, and it is for this reason that a mild sedative is of distinct aid in controlling the appetite in such patients. We have had only three patients who were given phenobarbital alone. The appetite was not altered in two, but it was definitely decreased in the third.

The fact that belladonna decreased the appetite in certain patients suggested that an alteration of motility of the stomach or small intestine may be a contributing cause. A series of investigations regarding this point have been in progress in this clinic, and the results will be given in another report. Lesses and Myerson[4] have reported that benzedrine will also diminish the appetite in certain patients. They state that this drug not only relaxes the stomach and intestinal tract, but also alters the mood of the patient and increases his sense of well-being. Atropine may possibly have some slight psychic effect in the doses administered in this study, but its principal effect is upon the gastro-enteric tract. These observations indicate that the action upon the gastro-enteric tract was the major one in decreasing the appetite of our patients with obesity.

SUMMARY

The administration of belladonna alone or with a mild sedative decreased the appetite in 40 of 45 patients. The relative number of patients affected undoubtedly would be less in a larger series of cases. The causes for the decrease in appetite are discussed.

REFERENCES

1. Greene, James A.: Clinical Study of the Etiology of Obesity, Ann. Int. Med. **12**: 1797, 1939.
2. Harrington, Mary M.: Appetite in Relation to Weight, J. Am. Dietet. A. **6**: 101, 1930.
3. Newburgh, L. H.: The Cause of Obesity, J. A. M. A. **97**: 1659, 1931.
4. Lesses, M. F., and Myerson, A.: Benzedrine Sulfate as an Aid in the Treatment of Obesity, New Eng. J. Med. **218**: 119, 1938.

TOXIC EFFECTS OF SULFANILAMIDE, SULFAPYRIDINE, AND RODILONE IN ANOXIA*

G. A. Emerson, Ph.D., D. W. Northup, Ph.D., and
E. J. Van Liere, Ph.D., M.D., Morgantown, W. Va.

EFFECTS of sulfanilamide on airplane pilots have interested Mackie[1] and Rook[2] in England, and their communications have received recent comment in the *Journal of the American Medical Association*.[3] Mackie reported that a pilot taking a full dose of sulfanilamide just prior to flying exhibited symptoms of anoxemia at an altitude of 13,000 feet. Since the partial pressure of oxygen at this altitude is 97 mm., this corresponds to only a moderate degree of anoxia. These authors state that sulfanilamide or its derivatives lowers the aviator's ceiling, or tolerance to anoxia, by about 5,000 feet. Rook[2] notes that similar observations have been made in America and Germany. Mackie[1] suggests that the cause of such reactions is an idiosyncrasy in which methemoglobin or sulfmethemoglobin is formed in sufficient amount to decrease significantly the oxygen-combining capacity of the blood.

Apart from the practical aspects of this purported effect of sulfanilamide derivatives, the problem deserves further investigation because cyanosis produced by sulfanilamide is not generally considered to be due entirely to inactivation of hemoglobin, and the effects of anoxia on actions and toxicity of sulfanilamide have not been thoroughly studied. Accordingly, the acute and cumulative toxic effects of three sulfanilamide-type drugs were proposed for study in mice exposed to various degrees of anoxia.

ACUTE EXPERIMENTS

From four to five hours after receiving oral doses of one of the three agents, or of the vehicle, 225 adult white mice, weighing from 18 to 22 Gm. each, were exposed to varying degrees of anoxia. All agents were suspended in 10 per cent aqueous solutions of acacia, and the concentrations adjusted so that all mice received 50 ml. per kilogram of solution. Sulfanilamide was given in a single dose of 2.0 Gm. per kilogram, and both sulfapyridine and rodilone were given in single doses of 10.0 Gm. per kilogram. Control mice were treated with 50 ml. per kilogram of 10 per cent acacia solution. Intragastric administrations were made with a blunted No. 18 needle, as recommended by Molitor and Robinson.[4] No deaths occurred immediately after treatment, showing that neither too rapid dilation of the stomach nor aspiration of solution into the lungs occurred.

Doses chosen were in the lower portion of the toxic range of the three compounds, for 3 of 64 mice treated with sulfanilamide, 3 of 59 treated with sulfa-

*From the Departments of Pharmacology and Physiology, West Virginia University School of Medicine, Morgantown.
Received for publication, November 18, 1939.

pyridine, and none of 53 treated with rodilone died during the four- to five-hour period prior to exposure to anoxia. None of 60 control mice which received acacia alone died before exposure to anoxia. Doses having a definite but low toxicity were purposely chosen to magnify any effects which anoxia might have.

Anoxia was produced in a decompression chamber resembling that of Kolls and Loevenhart.[5] This tank allows adequate ventilation, and even at very low pressures the air is changed rapidly enough to prevent accumulation of carbon dioxide. From ten to fifteen minutes were allowed for slowly decreasing the tank pressure, according to the final pressure attained.

Control mice, treated with acacia alone, were always exposed to low pressures simultaneously with mice treated with the drugs.

RESULTS

Four groups of 10 mice treated with sulfanilamide were subjected to anoxia for one hour. One group was exposed to a partial pressure of oxygen of 100 mm., the second group to a partial pressure of oxygen of 80 mm., the third group to a partial pressure of oxygen of 64 mm., and the fourth group to a partial pressure of oxygen of 53 mm. No deaths occurred at these levels, which correspond to approximate altitudes of 12,000, 18,000, 24,000, and 28,000 feet, respectively. All of 20 treated mice survived exposure to a partial pressure of oxygen of 48 mm., which is equivalent to an approximate altitude of 30,000 feet.

The same altitude equivalents and length of exposure were used in testing effects of sulfapyridine on susceptibility to anoxia. One of 10 treated mice died when exposed to a pressure corresponding to an approximate altitude of 18,000 feet, but 20 treated mice survived exposure to pressures equivalent to approximate altitudes of 24,000 or 28,000 feet. One of 15 treated mice died during exposure to a partial pressure of oxygen of 48 mm. (30,000 feet).

Parallel experiments were made with rodilone, except that no mice were exposed to a partial pressure of oxygen of 100 mm. Three of 10 mice died during exposure to a pressure corresponding to an approximate altitude of 28,000 feet. During exposure to a pressure equivalent to an approximate altitude of 30,000 feet, however, none of 20 treated mice died.

Groups of 10 mice treated with acacia alone were exposed to anoxia for one hour. One group was subjected to a partial pressure of oxygen of 100 mm., a second to a partial pressure of oxygen of 80 mm., a third to a partial pressure of oxygen of 64 mm., a fourth to a partial pressure of oxygen of 53 mm., and a fifth to a partial pressure of oxygen of 48 mm. In the last group a single death occurred.

Mice treated orally with 2.0 Gm. per kilogram of sulfanilamide showed marked prostration and slight convulsions. Degrees of anoxia used in this work had little effect on these symptoms. Mice treated with sulfanilamide were generally much quieter during the period of anoxia than were the control mice.

Treatment with 10.0 Gm. per kilogram of rodilone or sulfapyridine did not produce the prostration or convulsions observed in mice treated with sulfanilamide. Mice in these two groups were indistinguishable from the controls. It is notable that in these groups deaths occurred during anoxia, while no deaths occurred during anoxia in mice treated with sulfanilamide.

CUMULATIVE EXPERIMENTS

The three drugs and the vehicle were given orally, four times a day, for five days to four groups of mice before exposure to anoxia. Doses were spaced at approximately four-hour intervals, so that the mice received treatment for twelve hours each day. Since solution of these agents is slow, the actual time during which the mice were absorbing the drugs probably included at least sixteen hours each day. This procedure was adopted in order to follow as closely as possible the clinical use of these agents when intensive treatment is applied.

Also, single doses were low, although they were greater than the equivalent clinical doses on a weight basis. Doses used were below those which might be expected to produce any marked toxic symptoms. Sulfapyridine and rodilone were administered in single doses of 250 mg. per kilogram, corresponding to a daily dose of 1.0 Gm. per kilogram or a total dose of 5.0 Gm. per kilogram for the five days. Sulfanilamide was administered in single doses of 100 mg. per kilogram, corresponding to a daily dose of 400 mg. per kilogram or a total dose of 2.0 Gm. per kilogram for the five days.

All mice received these doses in single administrations of 0.1 ml. per 20 Gm. of solution, or a total of 100 ml. per kilogram of fluid in the five-day treatment period. The vehicle was 10 per cent acacia solution, and control mice received equivalent amounts of this. Since the mice voluntarily drank large amounts of water in addition to this, probably no disturbance in fluid balance occurred, although the daily amount of administered solution, 20 ml. per kilogram, is equivalent to the average daily urinary output in man. If hydration did occur, however, its possible effects on tolerance to anoxia are controlled, since the control mice received the same amounts of fluid.

Mice were weighed before the first treatment, on the third day of treatment, and on the sixth day, immediately before exposure to anoxia. The mean body weights of each group for these days were, respectively: for the controls, 21, 23, and 20 Gm.; for the group treated with sulfapyridine, 21, 23, and 20 Gm.; for the group treated with rodilone, 22, 22, and 20 Gm.; and for the group treated with sulfanilamide, 21, 20, and 19 Gm. Statistical examination of the standard errors of differences between these means revealed no significant changes. The slightly lower values on the sixth day were the result of a twelve-hour fast before exposure to anoxia.

RESULTS

On the sixth day, ten hours after the last treatment, the mice were exposed to anoxia for one hour. Twenty mice of each group were subjected to a pressure corresponding to an approximate altitude of 30,000 feet. One of the control mice which had been treated with acacia alone and 2 of the mice which had received rodilone died during anoxia. No deaths occurred during anoxia among mice which had been treated with sulfanilamide or sulfapyridine. At no time did any of the mice show the prostration or convulsions characteristic of acute sulfanilamide toxicity.

DISCUSSION

The results show that large oral doses of neither sulfanilamide nor sulfapyridine increase susceptibility to anoxia, nor do these agents exert a cumulative

effect which is detrimental to anoxic animals. This evidence might be expected from the success of the clinical use of these agents in pneumonia.

Results with rodilone are not sufficiently significant, when examined by the criteria of Loewenthal and Wilson,[6] to permit the conclusion that rodilone has any effect on tolerance to anoxia. Molitor and Robinson[4] comment on the marked cyanosis developed during repeated administration of rodilone. Although the careful studies of Harris and Michel,[7] and Long, Bliss, and Feinstone,[8] show the presence of methemoglobin or sulfmethemoglobin, or a fall in oxyhemoglobin content, in the blood of patients treated with sulfanilamide or sulfapyridine, it must be concluded from the present results that neither of these processes is important in mice treated intensively with any of the three compounds studied. Hematuria, if it occurred, was not sufficiently grave to produce an anemia severe enough to lower tolerance to anoxia.

The results may be considered confirmatory of Brun's[9] finding that these drugs do not increase the metabolic rate. Certainly there is no evidence of increased need of oxygen in the treated mice.

Of more practical importance than the present toxicity studies is the possible effect of sulfanilamide-type compounds on functions involved in precise orientation of persons. Rook[2] and Robertson[10] have indicated the possible dangers of allowing patients treated with sulfanilamide to engage in activities requiring precise judgment of orientation and speed. Experimental studies on man should be made to evaluate this point properly. In addition, the effect of the disease, for which sulfanilamide is being given, on judgment and coordination, must also be considered.

SUMMARY

Mice treated with single large oral doses of sulfanilamide, sulfapyridine, or rodilone showed no increased susceptibility to anoxia. Repeated treatment with smaller doses of these three agents given orally, four times a day, for 5 days also did not increase susceptibility to anoxia.

We are grateful to Dr. Hans Molitor, of the Merck Institute for Therapeutic Research, for generously supplying sulfapyridine (Dagenan) and rodilone (di-p-acetylaminophenyl-sulfone). All rodilone used was of the same preparation, designated as lot 144.

REFERENCES

1. Mackie, F. P.: Sulphanilamide in the Air: A Warning, Brit. M. J. **2**: 139, 1939.
2. Rook, A. F.: Sulphonamide in the Air, Brit. M. J. **2**: 306, 1939.
3. Foreign Letter, J. A. M. A. **113**: 1237, 1939.
4. Molitor, H., and Robinson, H.: The Acute, Cumulative and Chronic Toxicity of 2-Sulfanilyl Aminopyridine and di-(p-acetylaminophenyl)-sulfone, Arch. internat. de pharmacodyn. et de thérap. **62**: 281, 1939.
5. Kolls, A. C., and Loevenhart, A. S.: A Respiratory Chamber for Small Animals, Am. J. Physiol. **39**: 67, 1915.
6. Loewenthal, L. J. A., and Wilson, W. A.: Problems of Chance in Medicine and Research, Brit. M. J. **2**: 110, 1939.
7. Harris, J. S., and Michel, H. O.: Formation of Hemoglobin and Sulfhemoglobin During Sulfanilamide Therapy, J. Clin. Investigation **18**: 507, 1939.
8. Long, P. H., Bliss, E. A., and Feinstone, W. H.: Effects of Sulfapyridine, Sulfanilamide and Related Compounds in Bacterial Infections, Pennsylvania M. J. **42**: 483, 1939.
9. Brun, G. C.: Gibt es eine Stoffwechselsteigerung nach Zufuhr von Sulfanilamiden? Arch. internat. de pharmacodyn. et de thérap. **63**: 103, 1939.
10. Robertson, D. F.: Personal communication, Nov. 7, 1939.

INFLUENCE OF SODIUM CHLORIDE CRYSTALLIZATION BY CEREBROSPINAL FLUID*

SAMUEL BURACK, M.D., AND PAUL B. SZANTO, M.D.
KANKAKEE, ILL.

FOLLOWING the evaporation of a salt solution there results crystal formation which is specific for each salt and is the so-called crystal habit of the salt. Various workers have shown that the crystal formation of a salt may be easily affected by many factors. The minutest impurity in the salt solution will influence the crystal habit. Semenchenko and Shikhobalava[1] showed that the surface tension of the salt solution was the most important factor influencing the crystallization. Changes in the surface tension are reflected by changes in the crystal habit. These workers showed that the crystallization of sodium nitrate by evaporation is hardly influenced by substances which cannot change the surface tension of this salt. By the addition of surface-active substances a typical change of the crystal configuration is possible. Among the most important surface-active substances are albumin and globulin. These physicochemical facts were first used by Pfeiffer,[2] who noted that the addition of minute quantities of blood influenced the crystallization of a copper chloride solution. He further observed that blood from a tuberculous or cancerous patient gave a different type of crystal formation than did normal blood. He felt that by this method a serologic diagnosis of certain diseases was possible.

Tomesco and his co-workers[3] used the same principle for the study of the cerebrospinal fluid. They added minute quantities of cerebrospinal fluid to physiologic saline solution. They then studied the results of the crystallization by evaporation of this mixture at a temperature of 75° C.

The evaporation of 3 drops of physiologic saline solution results macroscopically in the formation of an outer ring surrounding an empty space. Microscopically, the sodium chloride crystals have a cubical shape. The addition of normal cerebrospinal fluid changes this picture. Tomesco and his associates found that the normal cerebrospinal fluid is able to change the crystallization in a dilution of 1:20 up to 1:60 or 1:80. Macroscopically, the evaporation of 3 drops on a slide shows a crystalline circumference enclosing a translucent layer. With higher dilutions this translucent layer disappears, and one sees only empty spaces, i.e., the same picture as seen without the addition of cerebrospinal fluid. Microscopically, the translucent area consists of rosettes, concentric circles, parallel lines, and needles.

*From the Kankakee State Hospital, Kankakee, Dr. G. W. Morrow, managing officer.
Received for publication, November 27, 1939.

In cases of general paresis Tomesco and his co-workers found typical changes in the crystal habit. Macroscopically, the central translucent layer was found to be present in dilutions up to 1:120. Microscopically, they found concentric circles, parallel lines, and fine needles, but the organization of the pattern was much finer than that of normal cerebrospinal fluid. The concentric circles around one or two cuboid sodium chloride crystals were especially finely organized. In all cases the cerebrospinal fluid showed positive Pandy and Nonne-Apelt tests, i.e., signs of increased protein content. In a later publication Tomesco and his collaborators stressed the fact that they found the so-called mixed form of crystallization in such mental diseases as schizophrenia and melancholia. This consisted of pictures which were between the normal and pathologic types. They felt that the increased protein is a very important factor but not the only one which can explain the reactions.

Finkelman[4] used the technique of Tomesco in a large series of cases. He examined 35 specimens of cerebrospinal fluid of patients with general paresis and found typical pathologic crystal patterns in 33 specimens. Of 17 persons with functional epilepsy, 11 showed normal pattern and 6 were of the mixed type. Of 16 persons with dementia praecox, 10 were normal and 6 were of the mixed type; in all these patients the Ross-Jones test was negative. Seventy-one specimens of spinal fluid from persons with nonneurologic disorders were all negative.

Recently Selzer[5] used Tomesco's technique but obtained different results. Although he found in the main in normal persons and in persons with pathologic disorders the same results as Tomesco, he is of the opinion that this method is without practical value because the formation of the pattern depends so much upon minute changes of the surface tension that sometimes drops from the same dilution evaporated on different slides, but at the same temperature, may show a different pattern formation. Selzer showed that there is an exact mathematical parallelism between the degree of the albumin content and the pattern formation.

The results obtained by these previous workers seemed of sufficient importance to us to prove the diagnostic value of this new reaction. In order to exclude any subjective factor the clinical diagnosis of the fluids examined was not disclosed to the examiner.

TECHNIQUE

We followed the method used by Tomesco and his co-workers and Finkelman. Briefly, this consisted of setting up ten test tubes of 0.8 per cent sodium chloride solution. The first tube contained 1 c.c., the second, 2 c.c., with increasing amounts up to 10 c.c. in the tenth tube. To each tube was added 1 drop of cerebrospinal fluid, and the tube was well agitated. Three drops of the contents of each tube were placed on separate slides, and the slides in turn were placed in an oven at 75° C., until the fluid was entirely evaporated. The slides, tubes, and pipettes used must be very clean and contain no impurities. The temperature is very important. We found that if the slides were kept at room temperature, or even up to 50° C., the crystallization shows a different picture.

The effect of other substances in influencing the crystal habit was shown by the following experiment: Using cerebrospinal fluid from a patient with general paresis, a typical organization pattern was obtained. To the same fluid in the test tube we now added 1 drop of an insulin solution; this led to the disappearance of the previous characteristic crystallization. The interpretation of the results is important. It seemed to us that the previous readings as positive, negative, or mixed type were not sufficiently exact. We also felt that the macroscopic picture had no diagnostic value in comparison to the microscopic picture. Normal or negative fluids show the following picture: Macroscopically, in a dilution of from 1:20 to 1:80 there is a translucent layer surrounded by a crystalline ring. This layer gradually disappears with higher dilutions. Microscopically, the translucent area consists of rosettes, concentric circles, parallel lines and needles (Fig. 1). In higher dilutions the pattern gradually disappears.

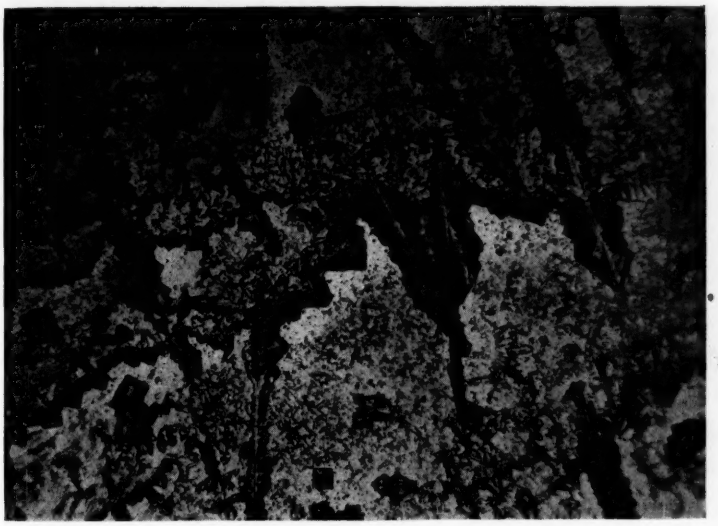

Fig. 1.—Crystallization of sodium chloride influenced by normal cerebrospinal fluid, dilution 1:80 (photomicrograph 1:80).

We divided the positive cases into three-, two-, and one-plus reactions. A three-plus fluid was characterized as follows: Macroscopically, there were no empty spaces in the translucent layer, even in a dilution of 1:200, i.e., 1 drop of spinal fluid plus 10 c.c. of saline solution. Microscopically, there were concentric circles, parallel lines, and fine needles, but the organization of the pattern was much finer and of a different nature (Fig. 2). The characteristic sign of a strongly positive pathologic fluid was the presence of a central cuboid crystal surrounded by many fine concentric rings. This is called good organization of the pattern and may be found in dilutions of 1:200 or even higher.

In a two-plus fluid the microscopic picture was the same qualitatively, but quantitatively there was a difference because the good organization was found up to only 1:140 or 1:160 dilution. The one-plus fluid shows a good organiza-

tion only in dilutions up to 1:100 or 1:120. As doubtful fluids we considered those cases where there was a more or less good organization up to 1:80. Sometimes one can find, even in normal spinal fluids in low dilution, a good organization pattern in the region just inside the peripheral crystalline ring. We do not consider this a pathologic finding.

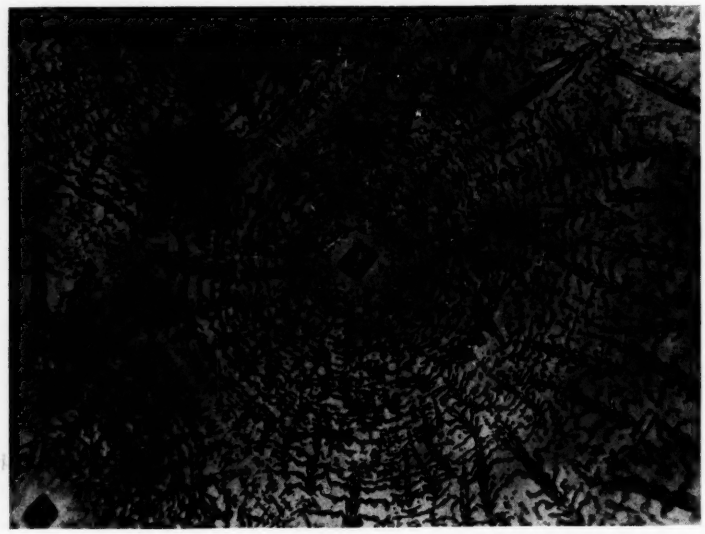

Fig. 2.—Crystallization of sodium chloride influenced by cerebrospinal fluid from a case of general paresis, dilution 1:200 (photomicrograph 1:80).

MATERIAL

We examined 263 persons. This consisted of 151 persons with general paresis, 46 with dementia praecox, 26 with chronic alcoholism, 8 with idiopathic epilepsy, 8 with mental deficiency, 4 with psychoneuroses, one with multiple sclerosis, one with pachymeningitis hemorrhagica interna, one with manic-depressive insanity, 8 with cerebral arteriosclerosis, and 9 without psychosis.

General Paresis.—Many of the 151 patients had been treated for varying periods of time, and some had completely negative serologic and spinal fluid findings. Of these, 31 gave a three-plus crystallization; 38, a two-plus; 46, a one-plus; 2 gave a doubtful and 34 a negative crystallization. In other words, the crystallization was positive in 115, or 76 per cent, of the cases.

Of more importance is a comparison between the crystallization finding and the other laboratory findings.

1. Of the 31 persons with a three-plus crystallization the blood Kahn test was positive in 27, and the spinal fluid Wassermann test was positive in all. The Lange colloidal test gave a typical paretic curve in 22 persons, a middle zone curve in 8, and a negative result in 1. The Ross-Jones test was positive in 12 persons, gave a trace of globulin in 12, and was negative in 7; i.e., this test was negative in 22 per cent of strongly positive crystallizations. The cell count was above 10 in 15 cases.

2. Of the 38 persons with a two-plus crystallization the blood Kahn test was positive in 27, and the spinal fluid Wassermann test was positive in 36. The Lange colloidal test gave a typical paretic curve in 4 persons, a middle zone curve in 30, and a negative result in 4. The Ross-Jones test was positive in 2 persons, gave a trace of globulin in 16, and was negative in 20; i.e., this test was negative in 52 per cent of the cases. The cell count was above 10 in 14 cases.

3. Of the 46 persons with a one-plus crystallization the blood Kahn test was positive in 30, and the spinal fluid Wassermann test was positive in 42. The Lange colloidal test was typical in one person, gave a middle zone in 33, and was negative in 12. The Ross-Jones test was positive in 2 persons, gave a trace of globulin in 10, and was negative in 34; i.e., this test was negative in 73 per cent of the cases. The cell count was above 10 in only 7 cases.

4. One of the two persons with doubtful crystallization showed a positive blood Kahn test; both showed negative spinal fluid Wassermann tests and negative Lange and Ross-Jones tests. The cell count was not increased.

5. Of the 34 persons with negative crystallization the blood Kahn test was positive in 7. Both the spinal fluid Wassermann and the Lange tests were negative in every case; in only one case did the Ross-Jones test show a trace of globulin and only in this case was the cell count above 10.

6. Of the 115 persons with positive crystallization the Ross-Jones test was positive in only 54, or 46 per cent of the cases. The Lange test was positive in 98, or 85 per cent of the cases. The spinal fluid Wassermann test was positive in 108, or 93 per cent of the cases. The cell count was above 10 in only 36, or 31 per cent of the cases. These results seem to indicate that the results of the crystallization show a parallelism first to the spinal fluid Wassermann test, and second, to the Lange test.

7. The crystallization was positive in every case in which the spinal fluid Wassermann test was positive, and in every case in which the Lange test gave a typical or middle zone curve. On the other hand, there were other cases in which the crystallization was positive, but the Lange, Ross-Jones, and spinal fluid Wassermann tests were negative.

Dementia Praecox.—We examined 46 persons with various types of dementia praecox. The crystallization was positive in 7 persons, doubtful in 4 and negative in 35. In the 7 persons with positive crystallization the blood Kahn test was positive in every case, the spinal fluid Wassermann test was positive in 4, the Lange test was of the middle zone type in 2, and the Ross-Jones test showed a trace of globulin in one case. In the doubtful cases the blood Kahn was positive in one case. In each one of the 7 positive cases syphilis was associated with the dementia praecox.

Chronic Alcoholism.—Of the 26 persons with chronic alcoholism one gave a positive and one gave a doubtful crystallization. No sign of syphilis was noted in either person.

Epilepsy.—Of the 8 persons with epilepsy one showed a two-plus crystallization. In this person the blood Kahn and the spinal fluid Wassermann tests were positive, and the Lange test was typical.

Mental Deficiency.—Of the 8 persons with mental deficiency 2 gave a doubtful crystallization, both of whom had positive blood Kahn tests but negative spinal fluid tests.

Psychoneuroses.—Of the 4 persons with psychoneuroses one gave a positive and one a doubtful crystallization. The person with the positive crystallization showed negative blood and negative spinal fluid tests, but his history stated that he had been treated for syphilis. The patient with the doubtful reaction had a positive blood test.

Cerebral Arteriosclerosis.—Of the 8 persons with cerebral arteriosclerosis two had positive crystallizations and positive blood Kahn tests but negative spinal fluid tests.

Without Psychosis.—All the remaining patients gave negative results.

COMMENT

A review of the preceding problem brought up the question of the factor or factors involved in the crystallization. Tomesco and his co-workers at first felt that the albumin-globulin content of the spinal fluid was the important factor because they noted that these substances are able to change the surface tension of the solution. In a later publication they stated that this cannot be the only factor, since they found strong pathologic or mixed type crystallization in persons with normal cerebrospinal fluid. Selzer stressed the increased albumin content as the only responsible factor. To prove this he showed that by removing the albumin of the spinal fluid the remaining fluid had no influence on the crystallization. By removing the albumin from normal and pathologic spinal fluids, as in general paresis, we got the same results as Selzer. However, we do not feel that we should draw the same conclusion as he did, i.e., that the only factor involved is the increased albumin, for it is possible to eliminate other unknown factors by removing the albumin. Perhaps besides the quantitative increase of protein in the spinal fluid, certain qualitative changes of the proteins are important, or a change in the albumin-globulin ratio may be responsible. From a practical point of view our results differed somewhat from those of previous workers. In case of general paresis our results were similar. However, in those cases of dementia praecox where we obtained a positive crystallization, syphilis was present in almost every case. In an occasional instance, alcohol seemed to be the only predisposing factor. It was also interesting to note in cases of general paresis that the crystallization phenomena seemed to be more sensitive than the colloidal gold curve.

We feel that further studies are indicated to discover the factors responsible for the crystallization phenomena.

SUMMARY

We examined the effect of normal and pathologic cerebrospinal fluid on the crystallization of sodium chloride. Spinal fluid of general paresis influences the crystal habit of sodium chloride in a typical manner.

REFERENCES

1. Semenchenko, V. K., and Shikhobalava, L. P.: The Effect of Impurities on Crystallization, Mineral Suir'e **11**: 27, 1936. (Chem. Abst. **30**: 7014, 1936.)
2. Pfeiffer, E.: Empfindliche Kristallisationsvorgänge als Nachweis von Formungskräften im Blut, Dresden, 1935, Emil Weise.
3. Tomesco, P., Cosmulesco, I., and Serban, F.: Une propriété inconnue du liquide céphalo-rachidien: la chloruro-cristallisation. Les aspects normaux, Bull. Soc. de psychiat. de Bucarest **1**: 11, 1936; La chloruro-cristallisation dans la paralysie generale. Les aspects pathologiques de cette propriété du liquide céphalo-rachidien, Bull. Soc. de psychiat. de Bucarest **1**: 38, 1936; La chloruro-cristallisation dans les maladies mentales non syphilitiques, Bull. Soc. de psychiat. de Bucarest **1**: 52, 1936; Formations cristallines du liquide céphalo-rachidien et chloruro-cristallisation, Bull. Soc. de psychiat. de Bucarest **2**: 122, 1937.
4. Finkelman, Isadore: The Effect Upon Sodium Chloride Crystal Growth of Contamination With Normal and Abnormal Cerebrospinal Fluids, J. LAB. & CLIN. MED. **23**: 1153, 1938.
5. Selzer, H.: Crystallization of Chlorides in Cerebrospinal Fluid, Arch. f. Psychiat. **107**: 775, 1936.

100 E. JEFFERY STREET

OBSERVATIONS ON THE INTERRELATIONSHIP OF CAPILLARY, PLATELET, AND SPLENIC FACTORS IN THROMBOCYTOPENIC PURPURA*

R. H. EGERTON ELLIOTT, JR., M.D., AND MARY A. WHIPPLE
NEW YORK, N. Y.

IN CASES of thrombocytopenic purpura a discrepancy between the degree of clinical bleeding and the level of the platelet count is not infrequently observed. One of us in a recent publication[1] was able to show that when this occurs the resistance of the capillaries, as measured by Dalldorf apparatus, tends to parallel the degree of clinical bleeding rather than the platelet level. In the same article attention was also called to the facts that following the performance of splenectomy for the relief of purpura, the capillary resistance rises with abrupt rapidity, that it often precedes in this respect the elevation of the platelet count, and that it may, therefore, be the first measurable sign of clinical improvement. In addition, it was concluded as a corollary that the suction test for capillary resistance was a useful method of measuring the activity of this disease and that in certain instances it was of more value in this connection than the enumeration of the blood platelets.

Since the publication of these investigations, we have been able to confirm the foregoing observations on numerous occasions. The number of consecutive cases of purpura which have been subjected to splenectomy and so studied in this clinic now totals 12. In every instance the results of our observations on capillary resistance have been essentially the same.

As a control to the latter observations, resistance determinations have been performed upon five individuals undergoing splenectomy for conditions

*From the Spleen Clinic of the Presbyterian Hospital and the Department of Surgery of the College of Physicians and Surgeons, Columbia University, New York.
Received for publication, November 27, 1939.

other than purpura. In only one instance was any alteration in resistance noted, and this proved to be a slow rise, quite unlike those observed in the purpura cases, in a patient whose spleen was removed to facilitate the excision of a pancreatic adenoma. In this instance, the change may well have been due to the general improvement of the patient following the removal of the tumor.

As additional controls the capillary resistance was followed in five individuals undergoing relatively simple abdominal operations in which the spleen was not removed. In none of these patients were the resistance values altered significantly.

To explain the relatively common phenomenon of the discrepancy between the platelet level and the intensity of the hemorrhagic manifestations in purpura, it becomes necessary to assume the presence of both a capillary and platelet factor in the mechanism of the disease. Bedson[2] gives the credit for advancing this hypothesis to Pierre Nolf, who offered the following ingenious experimental evidence in its support:

Using rabbits as subjects, he produced a marked, but transitory, thrombocytopenia by the intravenous injection of agar, and found that no purpura resulted. He then damaged the capillary endothelium of other rabbits by the intravenous administration of an anti-rabbit red blood cell serum. Again no purpura resulted, despite the fact that actual injury to the capillary endothelium could be demonstrated microscopically. When, however, he combined the two procedures, administering to the same animal first the anti-red blood cell serum and then the agar, i.e., when he added a thrombocytopenia to capillary damage, a marked degree of purpura resulted.

Inasmuch as our clinical investigations seemed not only to support Nolf's theory but also to establish the suction test as a relatively accurate method of measuring the severity of the disease process in purpura, we decided to repeat Bedson's experiments, hoping that by following the resistance of the capillaries we could better evaluate the individual importance of the capillary and platelet elements in the mechanism of this disease. In addition, it also seemed desirable to investigate the relationship of the spleen to these two factors. Therefore, for the sake of completeness and because of certain advantages which will be apparent later, we adopted the antiplatelet serum method of producing purpura as an alternative to the technique of Bedson described in the preceding paragraph.

TECHNIQUE

We employed then two separate and distinct techniques in the production of experimental purpura. They were (1) the antiplatelet serum method and (2) the method of Bedson. A brief description of each method is presented. If more detailed information is desired, the papers of some of the many investigators who have used essentially similar techniques may be consulted.[3-5] Light-skinned, male chinchilla rabbits, weighing between 2 and 3 kg., were used as the experimental animals throughout.

The Antiplatelet Serum Method.—In preparing the platelet antigen for the production of purpura by means of antiplatelet serum, the blood of two rabbits was drawn from the heart under nembutal anesthesia into an equal quantity of oxalated saline (sodium chloride 0.9 per cent and potassium oxalate 0.2 per cent). After centrifuging rapidly for one-half hour, the buffy coat was pipetted off and washed three or four times in normal salt solution to remove the remaining red blood cells as completely as possible. The yield, generally averaging between 1.5 and 2.5 c.c., was suspended in saline and injected intravenously into dogs. Four such injections were given at weekly intervals. A week after the last injection the dogs were bled and the desired quantity of antiplatelet serum was obtained. The serum so prepared was then injected intraperitoneally into rabbits, a dosage of 1.5 c.c. per kilogram of body weight being generally sufficient to produce a moderately severe thrombocytopenic purpura of from three to five days' duration (see Chart 1).

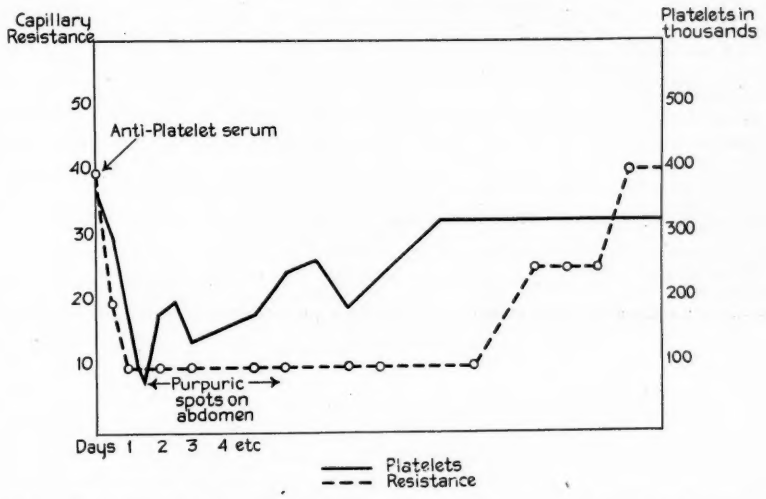

Chart 1.—Experimental thrombocytopenic purpura by means of antiplatelet serum showing the lag of the capillary resistance behind the platelets in returning to normal. Note also the relatively short duration of the purpura.

Bedson's Method.—The technique used in the preparation of an anti-red blood cell serum for the production of purpura by the previously outlined method of Bedson was essentially similar to that employed in the preparation of antiplatelet serum. At weekly intervals dogs were injected intravenously with a saline suspension of 2 c.c. of washed rabbit red blood cells, and a week after the fourth injection the desired amount of anti-red blood cell serum was obtained.

The agar-treated serum to be used in conjunction with the anti-red blood cell serum was made up as follows: One part of a suspension of 0.5 per cent agar in saline was mixed with four parts of fresh normal rabbit serum. The mixture was then incubated at 37.5° C. for two hours and allowed to stand at room temperature overnight. The following day it was centrifuged to remove any macroscopic agar and the clear supernatant serum was pipetted off. To produce purpura the anti-red blood cell serum was first injected intravenously into a rabbit, the dosage being 0.85 c.c. per kg. Three or four hours later the agar serum was administered to the same animal in similar fashion, the dosage in this instance being 5 c.c. per kilogram of body weight. The resulting purpura was to all appearances similar to that produced by antiplatelet serum, but more severe (Chart 2*a*).

OBSERVATIONS UPON NORMAL RABBITS

Fifty-six rabbits were used in the course of our investigative work. Upon 54 of these at least one or more control determinations were made. The determinations were carried out before subjecting the animals to experimental

procedures which would in any way affect their apparent good health. These determinations included hemoglobin values, red and white corpuscles, differential blood counts, bleeding and clotting times, clot retractility estimations, platelet counts, and capillary resistance determinations. This information is summarized in Table I, which includes the maximum, minimum, and mean values, together with the number of initial observations for each determination.

TABLE I

CONTROL VALUES

	NO. OF INITIAL OBSERVATIONS	MAXIMUM	MINIMUM	MEAN
Hemoglobin*	43	90	71	79
Red blood cell count	44	6,900,000	5,000,000	6,000,000
White blood cell count	42	12,100	5,600	9,100
Differential count†				
Polymorphonuclears	40	71	26	49
Lymphocytes	40	66	18	42
Monocytes	40	8	0	4
Basophiles	40	10	0	2
Eosinophiles	40	6	0	3
Bleeding time‡	41	4′00″	1′10″	2′14″
Clotting time	40	3′00″	0′45″	1′41″
Clot retractility§	22	24+ hr.	1½ hr.	
Platelets¶	54	438,000	178,000	298,000
Capillary resistance‖	33	50 plus	15	

*Sahli.
†Figures given in per cent.
‡Duke's method.
§In 7 cases there was no retraction after twenty-four hours.
¶Direct method.
‖Figures given are in centimeters of mercury negative pressure.

The figures in Table I for the hemoglobin content and cellular elements of the blood agree closely with those of the majority of other observers who have employed the same technical methods as were used here. Determinations of the bleeding time were not always satisfactory, as the test was done by pricking the marginal ear vein, the state of contractility of which was found to influence markedly the results in several instances. It was likewise found that estimations of clot retractility on the normal animal were of little use as control observations, for in a third of the animals upon which the test was performed, retraction of the clot was either delayed or failed to take place at all.

As in our clinical work, all capillary resistance determinations were done with the Dalldorf apparatus. The animals were shaved prior to testing, and the tests were carried out on the skin of the abdominal wall, the technique used being identical in every respect with that employed clinically.

The normal figures for capillary resistance in the rabbit were slightly higher than those we observed in human beings. In man we have found it ranged between 20 and 35 cm. of mercury negative pressure when taken on the forearm just below the antecubital fossa;[1] in the rabbit it ranged between 30 and 40 cm. of mercury negative pressure, with a mean value of approximately 35. The variation observed between rabbits tended to be greater than the variation between human beings, though an effort was made to minimize this discrepancy as far as possible by selecting light-skinned animals of the same

breed. The reason for this greater variation is due to the fact that greater individual differences exist in the texture, color, and thickness of the skin among rabbits than among human beings.

Two other factors that tended to influence the results of resistance determinations were shaving and the application of antiseptic solutions to the skin of the abdominal wall immediately prior to operative procedures. For this reason, when shaving was indicated, it was carried out twenty-four hours in advance of the performance of any determinations and iodine was generally omitted from the preoperative preparation of the skin, or if it was used, it was applied in weak dilutions, care being taken subsequently to remove it completely.

Despite the number of factors capable of influencing the results of this test, there was little variation between readings taken on different areas of the abdominal wall in the same animal, provided that the test was not performed on the flanks or in the groin.

Undoubtedly the suction test for capillary resistance is not as accurate in rabbits as it is in human beings. For this reason, we do not regard a change of resistance in rabbits as being of much significance unless it is greater than five points. Notwithstanding this, we feel that within broad general limits the test does give a reasonably accurate estimation of the state and change of resistance in the capillaries of the animals under discussion.

PROCEDURES

In our attempt to study experimentally the mechanism of the disease process in purpura, we pursued our investigations along lines as nearly parallel with our clinical studies as possible. With this in mind, the thrombocytopenic purpura produced by antiplatelet serum and Bedson's anti-red blood cell serum-agar serum methods was studied first. Subsequently, the effect of splenectomy both before and after the production of purpura by these methods was investigated.

PRODUCTION OF PURPURA

No difficulty was experienced in the production of purpura by means of either technique used. It was found that following the employment of these methods a thrombocytopenic purpura was produced which resembled the clinical form in every respect save that of duration, the experimental syndrome lasting usually no longer than five days. Of considerable interest was the fact that the capillary resistance which dropped along with the platelets generally required a much longer time to return to normal levels than did the latter (Chart 1).

Like Bedson, we found that the injection of anti-red blood cell serum alone, or of agar serum alone, did not produce purpura. In addition, these procedures had no appreciable effect upon the capillary resistance. We found, furthermore, that as a whole the animals subjected to Bedson's method developed a more severe grade of purpura than did those injected with antiplatelet serum. Charts 1 and 2*a* depict graphically the typical course of events in animals subjected to both of the afore-mentioned techniques.

SPLENECTOMY AND EXPERIMENTAL PURPURA

A. Operative Controls.—In order to parallel our clinical studies on splenectomy and to establish control groups for similar experimental investigations, the effect both of simple laparotomy and of splenectomy upon the blood values and the capillary resistance of normal animals was investigated. Ether anesthesia was employed in both procedures and the operative technique in each was identical, the only difference between the two operations being that in one the spleen was removed, and in the other it was exposed but not disturbed.

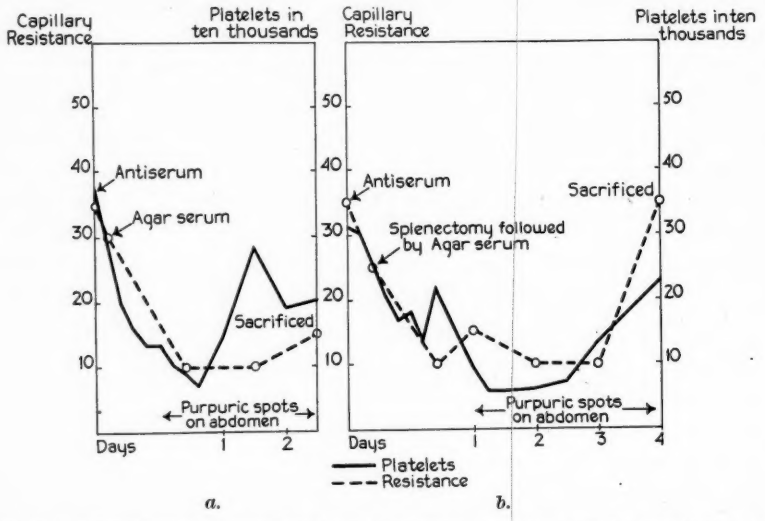

Chart 2*a*.—Experimental thrombocytopenic purpura by means of the combined administration of antiserum and agar serum. At autopsy this animal showed a very severe purpura with abundant hemorrhages into the mesenteries, the pancreas, and the subserosal tissues of the intestines. *b*, Experimental thrombocytopenic purpura following splenectomy performed during the combined administration of agar serum and antiserum. Autopsy showed severe purpura.

Following the performance of simple laparotomy, a slight rise in platelets was observed in each animal, the maximum and minimum increments for three animals thus followed being 96,000 and 19,000, respectively. The increments in the latter values following removal of the spleen, however, were considerably greater and averaged between 100,000 and 200,000 (see Chart 3*b*). In neither of these control procedures was the capillary resistance altered significantly.

B. Protective Effect of Splenectomy.—Bedson[7] has found that in guinea pigs splenectomy affords a varying degree of protection against the purpura produced by antiplatelet serum when this operation is performed prior to the administration of the serum. Because we wished to determine whether the same results obtained in rabbits and whether the performance of splenectomy before the injection of antiplatelet serum had any effect upon the capillary resistance, we repeated Bedson's experiment upon three animals.

Accordingly, the animals' spleens were removed, and six or seven days later, during the course of the ensuing platelet rise, antiplatelet serum was administered intraperitoneally. Despite the fact that the dose of the latter

was increased over the usual amount in all the animals and even administered in double the customary quantity in one, none of the rabbits developed purpura and none showed any change in capillary resistance (Chart 3*b*).

C. Splenectomy Following the Injection of Antiplatelet Serum.—The object of this group of experiments was to determine whether the removal of the spleen was as beneficial in experimental purpura as in the clinical form of the disease. Because of the relatively short duration of the purpura produced by the injection of antiplatelet serum, it was decided that it would be necessary to perform splenectomy early in the course of the disease. Therefore, as soon as a material drop in the platelet level and capillary resistance had occurred following the injection of the serum, the spleen of the animals was removed. It was realized that this was hardly a fair parallel to the circumstances obtaining when this operation is performed for the relief of purpura in man, and yet, for the reasons mentioned above, we could hardly do otherwise.

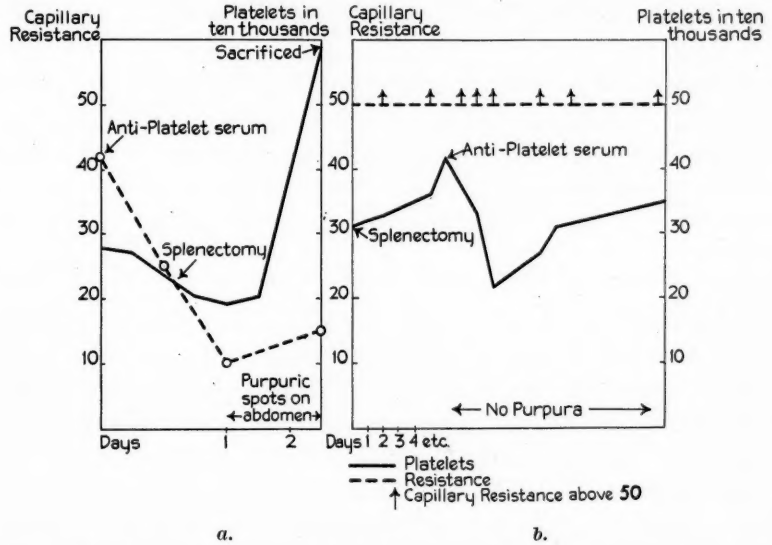

Chart 3*a*.—Experimental thrombocytopenic purpura following splenectomy performed after injection of antiplatelet serum. Autopsy on this animal showed moderate purpura. *b*, Protective effect of splenectomy performed prior to injection of antiplatelet serum. Note the absence of purpura and the continuance of the high capillary resistance level.

Four animals, then, were injected intraperitoneally with antiplatelet serum. Between two and three hours later splenectomy was performed, for it was generally found that material depressions in both capillary resistance and platelet values had occurred within this space of time.

In each of the 4 animals so treated, a purpura of varying intensity developed, despite the fact that in none did the platelet level drop below 170,000. The findings were corroborated at autopsy on each of the animals. As will be seen in Chart 3*a*, a fall in capillary resistance was produced which lagged well behind the platelets in returning to normal.

D. Splenectomy and Experimental Purpura Produced by Bedson's Method.—In these experiments the decision as to where in the course of the procedure to intercalate splenectomy was particularly difficult. In the first place, the

same handicap existed here as was met with in the preceding experiment with regard to the brevity of the purpura. In the second place, it was obvious from earlier experiments that if the operation was performed immediately following the administration of agar serum, the animals would in all probability not survive the trauma incident to operation, together with the rapid development of a severe purpura, if such should occur. Therefore, it was felt that if the operation were performed shortly *before* the injection of the agar serum, but sufficiently long *after* the administration of the anti-red blood cell serum to allow the latter to affect the capillaries, the animals would have a better chance of survival. In addition, by this procedure we would be able to determine whether splenectomy negated to any extent the capillary-damaging effect of the anti-red blood cell serum.

Accordingly, splenectomy was performed on three rabbits between two and three hours after the administration of the anti-red blood cell serum. As soon as the animals had recovered from the effects of the anesthesia, agar serum was injected.

Severe purpura developed in all the animals (see Chart 2*b*). Not only did splenectomy fail to alter the effectiveness of the anti-red blood cell serum, but as nearly as could be determined it in no way lessened the severity of the resulting thrombocytopenic purpura, the presence of which was confirmed at post mortem on each of the animals.

COMMENT

From the foregoing investigations it seems apparent that the administration to rabbits of either antiplatelet serum, or of agar serum in combination with anti-red blood cell serum, is an eminently satisfactory method of producing in these animals a thrombocytopenic purpura which closely resembles the disease as seen clinically. It is worth emphasizing, however, that the experimental purpura so produced is of short duration and, to all appearances, only in this respect does it differ from the clinical syndrome. In producing purpura by the combined administration of anti-red blood cell serum and agar serum, we were able to confirm Bedson's results with this method, which, it will be remembered, were offered as evidence in support of Nolf's theory that both a capillary and a platelet factor are present in the mechanism of this disease and are necessary to its production.

From the results of Bedson's work and our own, it seems apparent that when either a capillary-damaging factor in the form of an anti-red blood cell serum, or a platelet-reducing factor in the form of an agar serum acts alone, it is incapable of producing purpura. It is equally evident, however, that when these two factors act together in the same animal, purpura results. Furthermore, from our investigations we have been able to show that, acting alone, neither one of these two factors materially alters the resistance of the capillaries, but that acting collectively, they do.

We have shown that splenectomy in rabbits neither affects the individual potency of the afore-mentioned capillary-damaging and platelet-reducing agents,

nor alters their combined efficacy in the production of purpura. Furthermore, while we have confirmed and extended to rabbits Bedson's results on the protective effect of splenectomy performed prior to the administration of antiplatelet serum, we have, on the other hand, also been able to show that the performance of this operation following the administration of this serum affords little or no protection against the subsequent development of purpura.

In addition, our experiments have demonstrated that simple laparotomy and splenectomy in the normal rabbit do not affect the resistance of the capillaries, but do cause an increase in the number of platelets, which is of considerably greater extent in the latter operation than in the former. These observations lead us to feel that, as intimated by Bedson, the protective effect of splenectomy performed prior to the injection of antiplatelet serum is purely an indirect one and is due solely to the physiologic increase in the number of platelets which follows this operation. Additional confirmation of this would seem to lie in the fact that following the production of thrombocytopenic purpura by either the antiplatelet serum method or the agar serum method, and irrespective of whether the spleen was removed or not, the resistance of the capillaries undergoes a marked drop and in most instances does not return to its original value until well after the platelet count has resumed its normal level.

DISCUSSION

Though the thrombocytopenic purpura produced by the serologic methods herein described closely resembles in its manifestations the clinical form of this disease, there is no justification for assuming that the role of the spleen in the experimental syndrome is anything other than a minor one and of secondary importance. Certainly our experimental work, as well as that of Bedson, would seem to argue against such an assumption. On the other hand, the almost instantaneous rise in capillary resistance and the cessation of bleeding which follows removal of the spleen in idiopathic thrombocytopenic purpura is evidence that in the clinical form of the disease the role of the spleen is primarily an active one and of major importance. Further evidence for this contention is to be found in the fact that the afore-mentioned increase in the resistance of the capillaries and the alleviation of the hemorrhagic symptoms takes place too soon after operation to be accounted for by the subsequent increase in the number of platelets.

From our clinical as well as from our experimental work, we feel that we have definite evidence of the existence of both capillary and platelet factors in the mechanism of the disease process in thrombocytopenic purpura. In addition, we believe from our observations that the decrease in the resistance of the capillaries seen in this disease is probably the result of the combined action of these two factors. The fact that the capillary resistance tends at times to be dissociated from the platelet level makes us feel that the capillary factor is probably the more important of these two elements, if not in the initiation of the manifestations of the disease, at least in their maintenance.

SUMMARY AND CONCLUSIONS

1. A study of experimental thrombocytopenic purpura produced in rabbits by serologic methods has been made.

2. The relationship of splenectomy to the bleeding tendency, capillary resistance, and platelet count in thrombocytopenic purpura has been discussed.

3. In the absence of purpura, removal of the spleen in man and in rabbits does not affect the capillary resistance.

4. The suction method of measuring capillary resistance is a useful means of estimating the activity of the disease process in experimental as well as in clinical thrombocytopenic purpura.

5. It seems likely that both a platelet-reducing and a capillary-damaging element are present in the mechanism of the disease process in thrombocytopenic purpura and that both are necessary to the production of the disease.

6. The decrease in the resistance of the capillaries, which is seen in thrombocytopenic purpura, is probably the result of the combined action of the platelet and capillary factors.

7. Particular attention is directed to the importance of the capillary factor in thrombocytopenic purpura.

8. In experimental thrombocytopenic purpura produced by serologic methods, the role of the spleen is probably of secondary importance.

9. Evidence is advanced to support the contention that in idiopathic thrombocytopenic purpura the role played by the spleen is an active one and of major importance.

REFERENCES

1. Elliott, R. H. Egerton: The Use of the Suction Test for Capillary Resistance in Thrombocytopenic Purpura, J. A. M. A. **110**: 1177, 1938.
2. Bedson, S. P.: Blood-Platelet Antiserum. Its Specificity and Role in the Experimental Production of Purpura. Part II, J. Path. & Bact. **25**: 94, 1922.
3. Ledingham, J. C. G.: The Experimental Production of Purpura in Animals by the Introduction of Anti-Blood-Plate Sera; A Preliminary Communication, Lancet, June, p. 1673, 1914.
4. Ledingham, J. C. G., and Bedson, S. P.: Experimental Purpura, Lancet, Feb., p. 311, 1915.
5. Toeantins, Leandro M.: Experimental Thrombopenic Purpura in the Dog, Arch. Path. **21**: 69, 1936.
6. Dalldorf, G.: A Criterion of Hemorrhagic Diathesis in Experimental Scurvy, J. Exper. Med. **53**: 289, 1931.
7. Bedson, S. P.: The Effect of Splenectomy on the Production of Experimental Purpura, Lancet, Nov., p. 1117, 1924.

HISTAMINE THERAPY IN ALLERGY

LEON D. PORCH, M.D., MACON, GA.

A REVIEW of the literature relative to the use of histamine in the treatment of allergic disturbances indicates that this substance is perhaps one of the best available agents for alleviating the discomfort caused by these disorders, particularly skin conditions characterized by wheal and edema formation, erythema, pruritus, and the exudative diathesis. Recently, the role of allergy in peptic ulcer has been considered, and certain cases have been found to be associated with allergic manifestations. In this paper the therapeutic effect of histamine in urticaria and other skin lesions and in gastrointestinal ulceration will be evaluated.

Histamine was found in putrid soybeans by Yoshimura[3] in 1909, and was isolated from ergot in 1910 by Dale and Barger,[3] who also found it to be present in the intestinal mucosa. Chemically, histamine results from the decarboxylation of histidine, and it may be formed by (1) chemical decarboxylation, (2) bacterial decomposition, (3) ultraviolet radiation, and from (4) di-amino-acetone by chemical synthesis. Histidine is widely distributed in the body, being most abundant in such tissues as the intestines, liver, and lungs.[3]

In 1911 Dale and Laidlow observed that histamine may cause the typical symptoms and pathology of anaphylaxis when injected into normal animals. It caused a drop in blood pressure when injected intravenously into dogs, and it produced an increased capillary permeability or an edema. They showed, however, that the reaction of histamine differs from true anaphylaxis, in that histamine is still capable of producing strong reactions in the uterine strip even though the latter has been desensitized by treatment with specific serum. Lumière and Malespine[9] showed that the phenomenon of reciprocal protection from one shock by another shock, or anaphylaxis, extended equally to histamine accidents, suggesting an analogy in the mechanism of the two types of shock. These and other experiments revealed that histamine could cause in animals anaphylactic-like reactions which closely resembled allergic reactions, and the assumption was made that the formation of a histamine-like substance constituted a part of every allergic reaction.

In 1909 Eustis advanced a theory that asthma was produced endogenously by the absorption of a powerful toxin probably from the intestinal tract, and he suggested that histidine, or its histamine derivative, was so absorbed. Duke expressed the similar belief that allergic reactions may be caused by the absorption of a histamine-like substance from the intestines. Koessler[3] found, however, that histamine was rendered inert by its passage through the intestinal mucosa, and Wangensteen and Loucks[22] reported that the amount of histamine absorbed from a strangulated intestinal loop was very small.

Received for publication, April 9, 1940.

Making practical application of his theory, Eustis in 1912 began using histamine in small doses in the treatment of asthma. He reported beneficial results but stated that following injections of histamine several of his patients developed hives or nettle rash. This point is of signal interest in light of the more recent investigations of McConnell[9] and her associates who found that histamine, when applied to the skin of animals, or of man, promptly produces a wheal formation similar to that elicited by ordinary allergens in positive skin reactions. Lewis[3, 9] also has observed, in a study of the response of the skin and its blood vessels to a variety of stimuli, a reaction characterized by (1) local dilatation of minute blood vessels, (2) increased permeability of vessel walls by direct action, and (3) widespread dilatation of the neighboring arterioles through the medium of local reflex mechanism. He has shown that a similar reaction in the skin is produced by histamine, and he attributes this reaction to the effect of some chemical substance already present in the cells and perhaps released by the stimulus of histamine. In view of the histamine-like action of this substance, Lewis designated it the H-substance.

Kline, Cohen, and Rudolph,[9] in an investigation to determine the cellular reaction in allergic and nonallergic patients, found that specimens of tissue from allergic individuals excised after injections of allergens and histamine, and exposure to heat, cold, and scratching, showed almost complete infiltration by eosinophilic cells, whereas the reactions of nonallergic tissue were minimal. The histamine wheals in allergic persons were comparable histologically to those produced by allergens. This study indicates a similarity in the reactions of allergic individuals to ordinary allergens and to histamine.

It is apparent that the skin response to histamine application resembles that of allergen inoculation, and both in turn are similar to the wheal, erythema, and pruritus found in the lesion of urticaria or hives and in the bite or sting of bees.[19, 24] Apparently histamine or Lewis' histamine-like "H-substance" is present in all tissue cells, and, when reflexly stimulated, it acts by means of, or in conjunction with, certain as yet undetermined other substances to produce vascular disturbances. On the basis of this theory, the vascular response, according to its location, may be productive of hay fever, asthma, nettle rash, migraine, etc., including urticaria and other skin lesions, as well as peptic ulcer. Because of the histamine-like nature of the H-substance it appears reasonable to assume that repeated, small, increasing doses of histamine might reduce the tendency to vascular reactions (1) by acting as an antigen in conjunction with the tissue cells, (2) by desensitization, (3) by altering the chemical nature of the H-substance, or (4) by increasing the output of adrenalin or histaminase in the circulation.

The therapeutic efficacy of histamine in allergic disorders has been demonstrated by various investigators, who have reported its favorable use in asthma, migraine, lupus, pruritus, eczema, hypersensitivity to cold and to heat, periodic edema of the hand and angioneurotic edema, purpura, and other conditions including urticaria.[1-8, 10-13, 16, 18, 21, 25-27]

I. TREATMENT OF URTICARIA AND OTHER ALLERGIC DISORDERS

My use of histamine in 53 cases of urticaria and other skin lesions was stimulated by the report of Dzsinich,[4] who in 1935 reported complete relief in 12 of 15 cases of bronchial asthma and in 3 cases of urticaria after treatment with small doses of histamine. Following in general his outline of treatment, dosage beginning with 0.00001 mg. of histamine and increasing to 0.1 mg. was used, dilutions being prepared from 1 c.c. ampoules containing 1 mg., of histamine dihydrochloride (1:1,000 solution).* The initial dose was given intradermally and, as a rule, was 0.00001 mg. of histamine. If no reaction or aggravation of symptoms occurred following this dose, the same amount was injected subcutaneously forty-eight hours later, and the dose was increased according to schedule, doses being given not oftener than every other day. Any dose that appeared to aggravate symptoms was promptly reduced to one-tenth of the amount before increasing in accordance with schedule. (Dosages for treating urticaria and allied lesions in infants and children should be reduced in proportion.)

The solutions of histamine were prepared as follows.

1. Take 0.5 c.c. of the ampoule solution and dilute to 5 c.c. with normal saline. This gives 5 c.c. of solution containing 0.1 mg. of histamine per cubic centimeter.

2. Make subsequent dilutions similarly to obtain solutions containing 0.01 mg. per cubic centimeter, 0.001 mg. per cubic centimeter, and 0.0001 mg. per cubic centimeter, i.e., 0.5 c.c. of each immediately stronger solution, diluted to 5. c.c. with normal saline, will give the next weaker concentration, or one-tenth that of the respective "mother" solution. Solutions should be placed in appropriately labeled vials.

Each individual should first be tested with 0.1 c.c. from the vial labeled 0.0001 mg. per cubic centimeter, and the following considerations as to dosage should apply:

(a) Histamine-sensitive persons may experience headache, flushing of the face, and slight aggravation of symptoms. For this classification, the vial containing 0.0001 mg. per cubic centimeter should be further diluted *(0.5 c.c. diluted with normal saline to 5 c.c. and labeled 0.00001 mg. per cubic centimeter),* and graduated doses of 0.1 c.c., 0.3 c.c., 0.5 c.c., and 0.8 c.c. should be given from this dilution before proceeding with the routine schedule.

(b) All other cases should follow the schedule as given.

(c) For infants and children, test with 0.0000001 mg. or 0.000001 mg., making necessary dilutions similarly to those above.

Histamine was injected in most cases according to the following schedule, given at forty-eight-hour intervals for an average total of eighteen injections.

Vial 0.0001 mg./c.c.	0.1 c.c. (0.00001 mg.) intradermally
	0.1 c.c. (0.00001 mg.) subcutaneously
	0.3 c.c. (0.00003 mg.) subcutaneously

*The histamine preparation used in this study was Imido "Roche," manufactured by Hoffmann-La Roche, Inc., Nutley, N. J.

	0.5 c.c. (0.00005 mg.) subcutaneously
	0.8 c.c. (0.00008 mg.) subcutaneously
Vial 0.001 mg./c.c.	0.1 c.c. (0.0001 mg.) subcutaneously
	0.3 c.c. (0.0003 mg.) subcutaneously
	0.5 c.c. (0.0005 mg.) subcutaneously
	0.8 c.c. (0.0008 mg.) subcutaneously
Vial 0.01 mg./c.c.	0.1 c.c. (0.001 mg.) subcutaneously
	0.3 c.c. (0.003 mg.) subcutaneously
	0.5 c.c. (0.005 mg.) subcutaneously
	0.8 c.c. (0.008 mg.) subcutaneously
Vial 0.1 mg./c.c.	0.1 c.c. (0.01 mg.) subcutaneously
	0.3 c.c. (0.03 mg.) subcutaneously
	0.5 c.c. (0.05 mg.) subcutaneously
	0.8 c.c. (0.08 mg.) subcutaneously
	1.0 c.c. (0.1 mg.) subcutaneously

Patients notice a sensation of coolness in the skin following the first injection, and blanching of the skin is caused by peripheral capillary constriction. This is followed, within a few minutes, by diminution of edema, disappearance of wheals, and diminution of pruritic symptoms, with complete remission within a few hours, as a rule. However, symptoms return in about forty-eight hours after the first dose, although to a lesser extent. Subsequent doses apparently desensitize or increase the tolerance of patients; the result is complete and lasting remission of symptoms in the most responsive cases. The responsive patient suffering from urticaria with edema is comfortable after 3 doses, without recurrence of lesions.

My series of 53 patients with allergic skin with urticarial manifestations included 30 of urticaria alone, 22 of urticaria and angioneurotic edema, and one of pruritus without urticaria. Forty of these patients, or 75.5 per cent, were completely relieved by histamine without any additional therapy. Eleven persons, or 20.7 per cent, were improved, but other measures, including acid and enzyme (pancreas) administration, autohemotherapy, diet, removal of infection, etc., were necessary to complete relief. Two, or 3.8 per cent, were not benefited; one patient was aggravated by even minute doses of histamine, and the other had a shock reaction and was finally relieved by hysterectomy and autohemotherapy.

The duration of urticaria in these patients was from a few days to seven years, the average being four weeks to six months. The refractory period obtained through histamine therapy has lasted from twelve to twenty-two months. Only 3 patients have required additional therapy after twelve months, with ensuing benefit.

Other allergic syndromes in which histamine was employed included 5 cases of asthma, 2 of vasomotor rhinitis, 3 of infantile eczema, 3 of senile eczema, one of acute localized eczema of the forearms, three of atopic dermatitis, 3 of pollen dermatitis, 2 of pruritus ani, one of simple pruritus vulvae with edema, 2 of pruritus vulvae with leucorrhea, one of atrophic arthritis of the wrist, one of edema of the penis from ant bite, and one of hydroarthrosis of the knee, intermittent type.

SIDE REACTIONS

While small doses of histamine favorably influence the allergic disorder, larger doses aggravate paroxysms of asthma and also increase the intensity of urticarial symptoms. Final doses should not exceed 0.1 mg. or 0.1 c.c. of the original 1:1,000 solution. Toxic symptoms include headache, dizziness, profound weakness, flushing of the face to cyanosis, irritability of the nervous system, and dyspnea. Epinephrine is the natural antagonist of histamine and should be given if a severe reaction develops in any abnormally sensitive person.

Evidence of reaction may follow injection within a few minutes to several hours. In certain individuals a slight chilliness was experienced within fifteen to thirty minutes after injections, followed by headache, flushing of the face, drowsiness, nervousness, shortness of breath, and extreme weakness, lasting from a few minutes to, in one case, four days, during which time the patient did not feel able to get out of bed. As Brown has suggested, certain individuals manifest abnormal reactions to histamine; evidently after shock reactions a refractory period may follow.

In cases showing more than a mild reaction, subsequent doses should be reduced by one-tenth the original amount, given as mentioned previously. As a rule, untoward reactions do not follow reduction in dosage; instead there is definite improvement in symptoms. This being the case, histamine may then be given in increasing doses without fear of untoward accident. It has been noted that while initial minute doses may cause marked reactions in some cases, the tolerance of these patients apparently can be increased until large doses (approximately 0.1 mg.) produce no discomfort.

However, in view of the occurrence of a limited number of so-called toxic manifestations from small doses (0.00001 mg.), I feel it necessary to issue a word of caution against the indiscriminate use of histamine. It is apparently contraindicated in the presence of cardiac pathology, particularly in cases of myocarditis or coronary sclerosis.

CASE REPORTS

These selected case reports are representative of the favorable response to histamine therapy in urticarial lesions:

CASE 1.—R. A., 26-year-old male, a manufacturer of overalls, had had severe urticaria and sinusitis following undulant fever seven years previously. There was a history of allergy in his father's family. Both tonsils and adenoids had been removed. He had been a heavy drinker and stated that he had had urticaria almost constantly, especially after drinking. Physical examination was essentially negative. He gave positive skin tests to corn meal, rice, rye, cocoa, milk, chicken, asparagus, yeast, hops, all shellfish, and grass pollen (spring group). Treatment was begun with grass pollen desensitization and elimination diet. Sinusitis was relieved, and hay fever symptoms subsided, but urticaria was not benefited. Histamine therapy was begun, and, disregarding food tests, a full diet was given to combat a drop in weight. Doses from 0.000001 mg. increased to 0.5 mg. according to the outline of Dzsinich. Results were complete relief of urticaria, restoration of weight, stabilization of nervous system, and increase of energy. Six months later the patient once again began to take large quantities of alcoholic beverages without recurrence of urticaria except for one day. This occurred following a week's spree and was relieved by 0.00001 mg. of histamine intradermally.

CASE 2.—T. L., 31-year-old male, a railroad office assistant, had edema of the glottis, with generalized urticaria. He gave positive tests for a variety of foods and other substances and was subject to tingling of hands, face, and lower lip, followed by choking cyanosis and general urticaria. The first attack followed influenza. Physical examination was negative. He was given Rowe's diet for six months; his symptoms disappeared, and he was retested. His diet was changed; remission of symptoms followed for four months. A recurrent attack was relieved with 0.000001 mg. of histamine. The patient was desensitized with histamine and put on a regular, unlimited diet. There was no recurrence after four months, and the patient is in excellent health.

CASE 3.—F. W. G., male, an electrician, had acute urticaria and rhinitis of several weeks' duration. The patient's entire back was covered with brawny edema in which hard, marblelike areas could be palpated. Edematous area approximated 1 inch in thickness. Extremities were covered with macular-type urticaria with intense pruritus. Nasal discharge was clear but resembled that of hay fever in character. Moderate bronchitis was present. Treatment with small increasing doses of histamine was begun; total number of doses was 19. This was followed immediately by a gradual diminution of edema and disappearance of lesions. Body was clear within ten days. The patient has had complete relief for the past twenty-two months.

The foregoing case is typical of the majority of cases of urticaria relieved by histamine.

CASE 4.—T. T., male infant, aged 5 months, had acute infantile eczema of face, arms, and legs, and of body on ventral surfaces. There was a family history of allergy; the father had hay fever. The mother's milk did not agree with the patient so he had been fed with cow's milk formula since he was 2 months old. *No change in formula was made.* Histamine therapy was begun with a dose of 0.0000001 mg.; total number of injections was 11. Eczema disappeared in approximately five weeks. There was no recurrence within seventeen months.

This case is representative of the use of histamine in infants; similar decreased doses should be employed in other such cases.

CASE 5.—W. R. G., female, had severe pruritus ani and a history of hay fever, backache, and urticaria. Local examination by a proctologist revealed no pathology. Histamine was used for relief of intense pruritus; no local treatment was given to the rectum or the anus. A total of 19 injections, ranging from 0.00001 mg. to 0.1 mg. per dose, was given. This was followed by complete relief of itching symptoms and discomfort for thirty days, after which time symptoms returned.

Although relief in this case apparently was only temporary, it indicates the therapeutic value of histamine in this type of condition.

CASE 6.—R. E. B., female, for three years had had hay fever, asthma, and eczema (dry type) over the entire body as well as a tendency to angioneurotic edema and urticaria. She gave positive skin reactions to various foods and other substances. Diets and desensitization therapy were given for twelve months, with only incomplete relief from hay fever and recurrences of urticaria and eczema. Then histamine was given in doses of 0.00001 mg. intradermally to 0.1 mg. subcutaneously. As a result the skin is clear and apparently normal for the first time, and the patient notices only a slight early morning cough. Further treatment for pollen sensitivity is being given at present following completion of histamine therapy.

CASE 7.—M. M., school girl, had had itching for four months. Examination revealed scabies-like lesions with furunculosis from scratching and infection. Scabicides were used without relief. Intradermal histamine injection relieved itching within forty-eight hours, and histamine desensitization treatment was given. The result was complete relief.

DISCUSSION

The favorable results obtained in this series of cases bear out the reported effectiveness of histamine therapy in allergic manifestations. Use of histamine

in accordance with the desensitization schedule described would seem to offer a simple and reliable means of relieving skin disorders, in particular those disorders occurring as the result of an allergic reaction to external or internal irritants.

The lasting relief of conditions like urticaria afforded by a single series of histamine injections suggests that the administration of histamine effects a kind of desensitization at least comparable in results to that achieved by more laborious and more costly specific desensitization procedures and that it is often of greater duration than that obtained by the latter.

Successful relief of allergic disorders by histamine as reported lends support to the theory that histamine or a histamine-like substance is produced in the body as an intermediate step in the body's reaction to the majority of, if not all, allergens. Its production may be illustrated schematically as follows:

> Allergen → Sensitive body cells → Histamine (or histamine-like "H-substance") → Sensitive body tissue (vascular system) → Allergic reaction

Thus, on the basis of this hypothesis of the genesis of allergic reactions, when histamine is given according to a desensitization schedule, the sensitive body tissue, which formerly responded with a so-called allergic reaction to histamine produced by the impact of the allergenic substance on sensitive body cells, may be accustomed to histamine (desensitized) in such a way that it no longer reacts to the stimulus of allergen-produced histamine. The chain of events leading to the allergic manifestation is interrupted one step farther along than is the case with specific desensitization therapy where the response of sensitive body cells to the allergen itself is inhibited.

Obviously, the use of nonspecific histamine desensitization treatment has definite advantages which should appeal to both doctor and patient alike, since this method offers the doctor an immediate therapeutic means of relieving his patient, and also, in many cases, of obtaining a lasting remission, at a time when a search for specific irritants is exceedingly difficult. Of considerable importance is the fact that nonspecific desensitization therapy with histamine is considerably more economical than most specific desensitization procedures.

The side reactions reported are not uncommon to desensitization methods, and, while they illustrate the necessity for careful dosage and adequate supervision of the patient, they should not militate against the use of histamine any more than similar side effects deter the use of specific desensitization measures.

SUMMARY

Histamine dihydrochloride was employed parenterally in very small doses, increased from 0.00001 mg. to 0.1 mg. according to a definite desensitization schedule, in a series of 53 cases of urticarial-like allergic skin manifestations. Forty patients were completely relieved, 11 were improved, and only 2 were not benefited. Remission of symptoms obtained by histamine treatment has lasted for twelve to twenty-two months.

Twenty-eight other patients with various allergic syndromes were given histamine similarly, with a favorable response in the majority of instances.

These disorders, included infantile and senile eczema, rhinitis, pruritus ani, pruritus vulvae, atrophic arthritis, and edematous conditions.

Reactions of varying severity were experienced by some patients to injections of histamine. Reduction of the dosage in most cases effected control of the reaction and permitted effective continuation of therapy. However, careful administration of histamine is essential; its use is apparently contraindicated in patients with certain cardiac disorders.

CONCLUSIONS

Complete remission of symptoms may be obtained in many cases of urticaria by the injection of minute doses of histamine dihydrochloride, increased regularly by small amounts in accordance with a desensitization schedule.

The similar use of histamine may be beneficial in other allergic skin conditions, particularly those evidenced by pruritus, edema, and exudation.

A word of caution is indicated with regard to indiscriminate histamine therapy.

II. TREATMENT OF GASTRODUODENAL ULCERATION

The etiology of peptic ulcer is not yet definitely known, and various theories have been promulgated to account for its occurrence. Some of these hypotheses hold that an allergic reaction is at the etiologic base of many cases of gastroduodenal ulceration. The possible mechanism of such a reaction, involving liberation of a histamine-like substance and an ensuing vascular response which may eventuate in peptic ulcer formation, has been discussed in Part I.

Since desensitization with histamine was effective in correcting allergic manifestations such as urticaria and eczema, it was decided to employ a similar desensitization schedule in certain cases of peptic ulcer, on the assumption that peptic ulcer is caused by the influence of some allergen or allergens. This decision was aided by the fact that several patients treated for cutaneous allergic disorders simultaneously experienced relief of ulcer symptoms.

Eleven cases of peptic ulcer syndromes, in several of which the history suggested that allergy played a causative role, were given histamine injections, using the 1:1,000 solution of histamine dihydrochloride. The same schedule as for urticaria was followed—0.00001 mg. intradermally increasing to 0.1 mg. in successive subcutaneous doses at forty-eight-hour intervals. A twelfth patient with gastric carcinoma was also given histamine for its favorable effect on pain, appetite, etc. The brief case reports which follow illustrate the response of these 12 patients to histamine therapy.

CASE REPORTS

CASE 1.—L. D. E., male, aged 12 years, was sent in for an allergy examination to determine the cause of rhinitis. He had had an appendectomy two years previously, asthma the summer before, and head cold and sinusitis for the past few weeks. There was a history of allergy in the family and hay fever in the father. During examination the patient complained of pain in abdomen with marked tenderness over epigastrium. X-ray examination revealed characteristic ulcer deformity. Belladonna administration and a modified Sippy diet were begun. On the first day of his hospital stay an urticarial rash was noted over the abdomen, which itched intensely. A dose of 0.00001 mg. of histamine was given intradermally for the urticaria, and both urticaria and epigastric pain were relieved within twenty-four hours; previously it had been necessary to administer ⅛ gr. of morphine for

relief. Pain returned the third day, and histamine was again given, with astonishing relief. The patient was kept in the hospital for ten days on a modified Sippy diet and belladonna, but also received histamine according to the desensitization schedule every other day, with no recurrence of pain or other discomfort. After a six weeks' diet therapy x-ray showed an apparently normal intestine. There has been no recurrence within eighteen months.

Attention is called to the relief of urticaria and pain in this case.

CASE 2.—Negro, male, aged 50 years or more, a day laborer, complained of pain characteristic of duodenal ulcer. X-ray examination showed definite deformity of the cap; this deformity was not influenced by atropine. Sippy diet, belladonna, and sedatives were given for two weeks; the patient remained in bed for one week. This treatment did not bring about relief of pain and discomfort. Since it was evident that his condition was not improving and since it was difficult to keep him on a diet, surgery was considered. First, however, in view of the good results in Case 1, 0.00001 mg. of histamine was injected intradermally. The patient noticed almost immediately that his sensation of fullness was diminished, and he felt more comfortable and remained so for forty-eight hours; then symptoms returned. Subsequent small doses of histamine, increased every other day for approximately 20 doses to 0.1 mg., brought about apparently complete relief. Twelve months later, working twelve hours daily at hard manual labor and thinking very little about diet, this man is symptom-free.

Attention is called to the fact that the patient continued to experience fullness, soreness, acute pain, and distress until after histamine therapy was begun. From that point on there was a distinct diminution in his symptoms, with relative comfort after the fourth injection.

CASE 3.—J. L. P., male, aged 60 years, a railroad employee, had black tarry stools, extreme weakness, general gastric upset, and distress for one week before consulting me. I had attended him for perforations in the duodenum ten years previously, at which time an operation was performed which involved the simple closure of two ulcers with uneventful recovery. The patient was put to bed under treatment with an oral proprietary preparation, belladonna, and modified Sippy diet for one week. After stools had cleared, diet therapy was continued. X-ray examination showed constant deformity of ulcer, slight narrowing of pylorus, and retention after six hours. Medical treatment was continued, but as this did not give patient appreciable relief, gastroenterostomy was advised and declined. Mr. P. was convinced that he had a malignancy and would not listen to any suggestion of surgery, despite discomfort. He was finally persuaded to try histamine therapy, keeping a daily record of his sensations and symptoms following each dose. The following are excerpts from the record:

"5/11/38 At one-thirty P.M., after having taken a shot at eleven A.M., am feeling fairly comfortable in my stomach.

"5/13 Had no unpleasant feeling in my stomach after lunch today. Lay down for a couple of hours, got up feeling better than for a couple of weeks.

"5/15 Did not wake with pain last night as usual.

"5/17 Had real severe pain last night but ate a canned pear which I think caused the trouble.

"5/19 I am feeling lots better after each shot now, only I am growing a bit weak.

"After the fourth shot the only change I notice is that I am feeling better each day. . . . I did not keep further daily data as the only effects I felt were that I was improving daily. I would have turned this in sooner but was waiting to see what the results would be and to see if the shots had made a complete cure, which I am convinced they have, as I have had absolutely no pain since the fourth shot. My appetite is good, and I eat practically anything I want, but I try not to eat too much. Have lost only one day from work since the thirteenth of August and this is the twenty-eighth of September."

These results indicate the evident relief with histamine, without observation of dietary restrictions. Several weeks later, however, x-ray examination indi-

cated a defect which, I believe, may be the result of adhesions following previous perforation, since the patient is symptom-free.

CASE 4.—B. A., white male, aged 37 years, complained of duodenal ulcer. He stated that he felt exactly as he did prior to his last perforation—he had been operated upon twice previously with simple closure of perforations. Physical examination was essentially negative except for ulcerlike tenderness and marked undernourishment. There was a history of two attacks of asthma in 1934, with occasional difficulty in breathing and nasal obstruction. Since the patient required considerable building up for operation and since there was no indication of pyloric obstruction, he was put on an oral proprietary preparation, belladonna, and a modified Sippy diet, with occasional sedatives. After three weeks he did not improve subjectively and histamine therapy was initiated, beginning with 0.00001 mg. intradermally. There was a noticeable improvement in comfort during the next forty-eight hours. Histamine injections were continued, with relief of symptoms, and the patient was discharged some three weeks later, apparently well and symptom-free. After a short interval, during which time no injections were given, symptoms returned, and a posterior gastroenterostomy was performed. The stomach was normal and the duodenum showed two healed, old ulcers, but no evidence of induration of recent ulcer. Adhesions in the upper gastrointestinal tract were corrected. Following the operation recovery was uneventful except for hiccough which responded to treatment, but convalescence was delayed by the patient's mental state. He would not take nourishment, complained of every trifle, and, in general, was very hard to manage. Since such notable improvement had resulted from histamine prior to operation, it was again employed in an attempt to aid in recovery. Injections of small doses brought about a decided change for the better: the patient's appetite returned, his attention to personal appearance improved, and he wanted to sit up and get out of bed. Saline injections were given for one week as a control procedure and symptoms returned, to be relieved by further histamine therapy.

In this case the preoperative use of histamine enabled us to build up the patient sufficiently to stand operation, and its postoperative use was of benefit in his recovery. Gastroenterostomy described above was performed in September, 1938; in April, 1939, the patient complained of soreness and distress in epigastrium. He was again given 13 injections of histamine, following which his symptoms subsided and he gained 6 pounds. The patient is apparently well at present.

CASE 5.—H. D., male, aged 40 years, complained of pain in stomach, gas, vomiting, blood in stools, and fullness after meals. Physical examination revealed an undernourished male, with tenderness in epigastrium. X-ray examination showed normal esophagus and stomach; a large crater on posterior wall of the duodenal bulb, a wide inflammatory ring about ulcer, and a diverticulum on the descending portion of duodenum. The patient was given 0.00001 mg. of histamine and on the following day felt better than he had for three weeks previous. A bland diet was given without alkali neutralization or other medication. After two injections of histamine the patient was discharged from the hospital under the care of his physician who continued with a regular schedule for histamine injections. He improved clinically from the first injection. Two months after histamine therapy was begun, x-ray examination showed a fluoroscopically normal stomach, with no hypersecretion; moderate constant deformity of duodenal bulb, but no visible niche; no retention at six hours, but moderate degree of hypermotility. His physician reports clinical relief has been maintained to date.

CASE 6.—E. McK., female, aged 41 years, had pain in epigastrium and distress characteristic of ulcer. She had been treated for peptic ulcer since October, 1938, at which time she had a hemorrhage. Two years previously a gastroenterostomy had been done. Physical examination was essentially negative except for tenderness in epigastrium to left of old laparotomy scar. X-ray examination on admission revealed evidence of a stomal ulcer with

a well-functioning gastroenterostomy. After a blood transfusion the patient was put on histamine therapy and a bland diet, with apparent clinical improvement. She was given injections at the hospital for ten days and then discharged; she received subsequent injections at the office. She was apparently symptom-free one month after beginning histamine therapy, and although she had slight retention at six hours, the roentgenologist reported that no stomal ulcer could be demonstrated. After a remission of several weeks, the patient again complained of distress in epigastrium. X-ray examination check showed a well-functioning gastroenterostomy; a small niche was observable at the stoma. Barium did not pass through the duodenum, there being a 10 per cent retention at six hours; the remainder of the barium passed normally into the cecum. The patient was advised to re-enter the hospital for observation, but a bed was unavailable at the time and she has since failed to appear for treatment.

In this case definite clinical improvement was noted, particularly subjective improvement, regardless of the x-ray findings. The patient was almost deaf, and it was exceedingly difficult to obtain information from her; also her mental state was not the most favorable for this type of cooperative study.

CASE 7.—C. B. A., female, aged 48 years, complained of pain in the right upper quadrant and back, with hematemesis, indigestion, fullness in epigastrium, headache, sinusitis, nervousness, and hot flushes. General examination revealed only tenderness over the gall bladder on deep pressure; symptoms and history were suggestive of gall bladder disease or peptic ulcer. X-ray examination showed good gall-bladder shadows, with no evidence of calculi; in gastrointestinal series the stomach appeared normal, with normal peristalsis and well-filled duodenal bulb. There was a minimal deformity of the duodenal cap, without definite niche. The patient had a chronic duodenal ulcer and was put on a bland diet without antacid medication and allowed to be ambulant. A daily record of experiences following histamine injections was kept. After the sixth dose she began to feel comfortable and did not spit up further blood. She received from 0.00001 mg. to 0.1 mg. of histamine in 17 injections.

I was unable to again x-ray this patient after the period of histamine injections, so no definite conclusion can be made of the degree of healing of her ulcer which may have taken place. Following the sixth injection, however, she was clinically free of symptoms for an indefinite period of time, slight symptoms recurring after twelve months.

CASE 8.—F. L. E., male, aged 31 years, a painter, had pain in his right side, nagging in character, with nausea, occasional vomiting, constipation, and nervous indigestion for six years. Physical examination was essentially negative except for poorly kept teeth and pyorrhea. X-ray examination after barium enema showed no evidence of pathology. In gastrointestinal series the chest and esophagus were fluoroscopically normal, there were considerable hypersecretion in stomach, spastic pylorus, marked deformity of duodenal bulb, and definite niche; the second portion of the duodenum was redundant. There was 20 per cent retention in stomach at six hours, but progress of the barium was normal beyond the stomach. The patient was put on a bland diet, and injections of histamine were begun. Immediately following the first dose (within three to four hours), he experienced definite relief of sensation of fullness, gas, and pain in the side; his appetite improved, and he felt generally better and stronger. Despite a complete series of histamine injections he still continued to experience some discomfort in his side. A confirmatory x-ray examination was made; it showed no abnormality in esophagus or stomach, but revealed a large ulcer pocket just distal to the pyloric sphincter. Retention in the stomach with considerable spasm about the pylorus was also noted.

In this case a combination of aluminum hydroxide, diet, rest, and histamine would probably have effected a more definite response. Subjectively, this

patient was the only one of those with known ulcer who did not respond satisfactorily to histamine alone. However, both x-ray and gastric analysis indicated improvement.

CASE 9.—J. M. G., male, aged 41 years, has since 1929 suffered from gastrointestinal symptoms suggestive of duodenal ulcer. He has taken soda, mineral oil, etc., for distention, gas, and discomfort most of his life. An x-ray examination in 1936 showed that he had an ulcer. A second x-ray examination in 1937, by a different laboratory, did not demonstrate an ulcer. He was given 14 injections of histamine (0.00001 mg. to 0.01 mg.) at intervals of every other day; this therapy resulted in remission of symptoms for seven months, after which time he returned for further prophylactic injections, stating that for this period he had been symptom-free.

CASE 10.—O. G. C., male, aged 46 years, was x-rayed in 1937 and told he had an ulcerated stomach. An x-ray examination in 1939 revealed a normal stomach and upper intestinal tract, but definite colitis and symptoms of duodenal ulcer. Because his teeth and gums were poor, his teeth were removed. Histamine injections were given before and during the removal of teeth with lasting abatement of symptoms. The discomfort was relieved immediately and has not returned to date.

CASE 11.—W. I. G., male, aged 40 years, was previously x-rayed, but no definite evidence of ulcer was found. He presented a history of indigestion, gas distention, nausea, gastric upsets, constipation, relief from soda, etc. A series of histamine injections afforded him remission of symptoms for several months. At present, after a nine months' symptom-free period, he is again experiencing some discomfort.

CASE 12.—J. W., aged 39 years, was admitted to service on Aug. 23, 1939, with diagnosis of gastric ulcer. He was discharged and re-admitted on Sept. 20, 1939. He gave a history of syphilitic infection fifteen years previously with requisite treatment. X-ray examination revealed advanced infiltration of carcinoma in cardia and lower esophagus. The patient was, therefore, not a proper subject for histamine therapy, but histamine was given in an attempt to improve his appetite, and to determine what effect, if any, it might have on his feeling of well being. A noticeable improvement in comfort, in appetite, and in mental outlook resulted. The patient became hungry and had no difficulty with his food. His general condition was also improved considerably prior to his departure to a clinic for surgical attention.

DISCUSSION

It may be very significant from the standpoint of etiology of peptic ulcer that the same dosage schedule of histamine injections employed successfully in treating urticaria and other skin lesions is effective in affording remission of ulcer symptoms. An allergic reaction has been proved to underlie the urticarial disorders described in Part I, and reasoning by analogy one may assume that cases of peptic ulcer responsive to histamine are similarly caused and perpetuated.

Of course, it is not justifiable to assume on the basis of results in this small initial series that all cases of peptic ulcer have an etiologic background of allergy; in fact, the effectiveness of histamine may conceivably be that of the substance per se, rather than of its desensitizing influence against external or internal irritants. However, there is sufficient evidence of improvement of ulcer symptoms in individuals of an allergic diathesis after injections of histamine to warrant the assertion that allergy plays a certain causative role. This assumption derives further support from the observation of simultaneous relief of allergic skin disorders in patients whose peptic ulcer symptoms are also benefited.

Psychic factors should be considered to a certain extent in evaluating the efficacy of this type of therapy. However, that they probably play a minor role is evident from the finding that in at least one case, symptoms which had been relieved by histamine injections returned when saline solution was substituted for the histamine; when histamine therapy was reinstituted, the symptoms once more disappeared.

The small doses of histamine injected did not stimulate gastric acid secretion, as demonstrated by gastric analyses. This finding is of importance in view of the fact that peptic ulcer is invariably aggravated by increased acid flow. An effective therapeutic method should have no stimulating influence on acid production, and histamine therapy in the dosage employed apparently satisfies this criterion.

SUMMARY

Eight patients with peptic ulcer syndromes and demonstrable ulcers were treated with a bland diet, supplemented by injections of hitamine according to a desensitization schedule, without any attempt at acid neutralization. Similar treatment was given in three other patients with similar symptom syndromes but without demonstrable evidence of ulcer. A twelfth patient with advanced carcinoma of the esophagus and stomach was also given a series of histamine injections during his hospitalization period.

Subjectively, the ulcer patients experienced relief of symptoms, in most instances following the fourth histamine injection, and they were able to enjoy a fuller diet than the routine in similar cases. There was a return of a sensation of well-being in the person with cancer, with increase in appetite as well as in comfort. Remission of symptoms in duodenal ulcer syndromes without demonstrable lesions was apparently dependent on the total number of injections and the amount of histamine given.

Studies of gastric function did not demonstrate hypersecretion of acid from small doses of histamine; rather, there appeared to be some inhibition of secretory activity, and in one case actual lowering of the acid curve.

X-ray studies of gastric functional activity and the regression of previously demonstrated niches in several cases confirmed subjective evidence of improvement or healing of ulcer lesions.

No marked reactions to injection of histamine, but occasional headache and generalized aching, with a feeling of weakness, were experienced by some patients.

CONCLUSIONS

It would appear from the results obtained and the observations made in this small series of cases that repeated injections of small amounts of histamine, in increasing doses, can well be used to augment the more recognized treatment of peptic ulcer, supplementing diet, rest, and acid neutralization.

It would also appear that the use of histamine desensitization therapy in patients with ulcer symptom syndromes, but without demonstrable ulcers, can afford these patients a remission of symptoms.

The favorable response of patients with peptic ulcer syndromes to histamine in small increasing doses would indicate an allergic basis for the develop-

ment or maintenance of the syndrome in these cases. The response is similar, in respect to the type of therapy involved, to that observed in cases of urticaria and other skin lesions of a proved allergic etiology.

From a consideration of the various factors involved, it would appear that the use of histamine in peptic ulcer has a certain place, particularly in those patients who are of the so-called allergic diathesis type and manifest other symptoms of an allergic character.

Further investigation of the histamine therapy of peptic ulcer is recommended.

Acknowledgment is hereby made to Dr. Alvin E. Siegel and Dr. B. S. Gostin for the use of cases from the Medical Services of the Macon Hospital and for their interest and assistance.

REFERENCES

1. Editorial note: Antihistamine Therapy and Treatment of Asthma, J. A. M. A. **100:** 280, 1933.
2. Auld: Treatment of Asthma, Brit. M. J. **1:** 171, 1928.
3. Bray: Recent Advances in Allergy, ed. 3, Philadelphia, 1937, P. Blakiston's Son & Co.
4. Dzsinich: Histamine in Treatment of Allergic Conditions, Klin. Wchnschr. **14:** 1612, 1935; Abstr., J. A. M. A. **105:** 2191, 1935.
5. Ernstene and Banks: Histamine Therapy of Pruritus; Preliminary Report, J. A. M. A. **100:** 328, 1933.
6. Fiessinger and Gajdos: Reactogenotherapy of Recedivating Urticaria Ionization, Presse méd. **43:** 1913, 1935.
7. Friedlaender and Petow: Migraine as Symptom of Biliary Disease; Treatment With Histamine, Med. Klin. **23:** 1498, 1927.
8. Gajdos: Reactogen Therapy by Histamine Ionization in Recurrent Urticaria, Rev. gén. de clin. et de thérap. **48:** 517, 1934.
9. Hansel: Allergy of the Nose and Paranasal Sinuses, St. Louis, 1936, The C. V. Mosby Co.
10. Israel, Jacob, and Meyer: Persistent Urticaria Treated by Cutaneous Scarification With Histamine, Strasbourg méd. **96:** 43, 1936.
11. Meineri: Morphine, Histamine, and Peptone in Therapy of Lupus, Dermosifilografo **6:** 185, 1931.
12. Stahl and Masson: Histamine; Cutaneous Application in Asthmatiform Dyspnea, Compt. rend. Soc. de biol. **111:** 1003, 1933.
13. Thiberge: Histamine Therapy of Asthma, New Orleans M. & S. J. **85:** 39, 1932.
14. Wise and Sulzberger: Yearbook of Syphilis and Dermatology, 1936.
15. Tuft: Clinical Allergy, Philadelphia, 1937, W. B. Saunders Co.
16. Saylor and Wright: Studies on Two Cases of Urticaria From Cold Sensitivity and of the Effect of Histamine Treatment, Am. J. M. Sc. **192:** 388, 1936.
17. Collens, Lerner, and Fialka: Insulin Allergy; Treatment With Histamine, Am. J. M. Sc. **188:** 528, 1934.
18. Kling and Sashin: Histamine Iontophoresis in Rheumatic Conditions, etc., Arch. Phys. Therapy **18:** 333, 1937.
19. Bloomfield and Pollands: Studies of Gastric Secretion, J. A. M. A. **92:** 1508, 1929.
20. Bartosch: Histamine in Anaphylaxis in the Guinea Pig, Klin. Wchnschr. **14:** 307, 1935.
21. Dzsinich and Paul: Treatment of Allergic Disorders, Klin. Wchnschr. **15:** 1754, 1936.
22. Wangensteen and Loucks: Studies in Intestinal Obstruction; Absorption of Histamine, Arch. Surg. **16:** 1089, 1928.
23. Essex, Markowitz, and Mann: Physiologic Action of Bee Venom, J. Applied Physiol. **94:** 209, 1930.
24. Benson and Semenov: Allergy in Relation to Bee Stings, J. Allergy **1:** 105, 1930.
25. Mueller: Periodic Edema of the Hand With a Seven-Day Cycle; Treatment With Histamine, M. Clin. North America **13:** 1543, 1930.
26. Bray: A Case of Physical Allergy; Localized and Generalized Allergic Type of Reaction to Cold, J. Allergy **3:** 367, 1932.
27. Horton: Hypersensitiveness to Cold, Proc. Staff Meet., Mayo Clin. **9:** 477, 1934.
28. Eusterman and Balfour: The Stomach and Duodenum, Philadelphia, 1936, W. B. Saunders Co.

CLINICAL CHEMISTRY

THE ACID-BASE BALANCE IN THE PLASMA AND BLOOD CELLS OF NORMAL NONPREGNANT, PREGNANT, AND PUERPERAL WOMEN*

FRED W. OBERST,† PH.D., LEXINGTON, KY., AND E. D. PLASS, M.D.
IOWA CITY, IOWA

THE acid-base equilibrium in the plasma of normal pregnant women has been intensively studied by many investigators, but there is still some disagreement concerning the extent and significance of the variations involved. Oard and Peters,[1] Muntwyler, Limbach, Bill, and Myers,[2] Kydd,[3] Kydd, Oard, and Peters,[4] Myers, Muntwyler, and Bill,[5] and Nice, Mull, Muntwyler, and Myers[6] have recently reviewed the literature and have made excellent contributions toward the solution of this complex problem. It is generally agreed that during gestation there is a reduction in the carbon dioxide content and in the protein percentage of the plasma, which is associated with a comparable diminution in the total base, but there is still some difference of opinion as to whether these changes affect the hydrogen-ion concentration. The present study was designed to investigate the plasma and whole blood and by calculation to determine alterations in the blood cells.

MATERIAL

Complete studies were made on (a) ten normal nonpregnant women of the childbearing age (nurses and ambulatory patients with minor afflictions that ordinarily would not disturb the acid-base or the water balance), (b) ten primigravidas and ten multigravidas who were in the third trimester of pregnancy and who showed no signs or symptoms of disease, and (c) ten normal postpartum women who were tested seven to nine days after delivery. All individuals received the regular (mixed) hospital diet.

METHODS AND CALCULATIONS

A portion of the blood was drawn under anaerobic conditions into tubes containing dry sodium oxalate and oil and was analyzed for carbon dioxide content and capacity, oxygen content and capacity, chlorides, phosphates, cell volume, and water. A second portion, similarly treated, was centrifuged under oil, and the supernatant plasma was used for determining the carbon dioxide content and capacity, hydrogen-ion concentration, total protein, nonprotein nitrogen, chlorides, phosphates, and water. The final portion was collected in a clean dry tube and allowed to clot, the serum being used for analyses of sodium, calcium, magnesium, and sulfates. Potassium was determined on the solid

*From the Department of Obstetrics and Gynecology, State University of Iowa.
†Biologic chemist at the United States Public Health Service Hospital, Lexington.
Received for publication, February 14, 1940.

residues obtained when whole blood and plasma were dried to constant weight in the water content determinations, as previously described.[7]

The concentrations of the various blood cell constituents were calculated from determined data on the concentrations in whole blood and plasma and on the cell volumes, using the generalized formula:

$$\text{Concentration in the whole blood} = \frac{\text{Concentration in the plasma} \times \text{plasma volume}}{100} + \frac{\mathbf{X} \times \text{cell volume}}{100},$$ where **X** is the concentration in the cells.

The various plasma and cell constituents are reported in terms of milliequivalents.* The total acid represents the summation of the anions bound with the basic elements. Total acid equals $[\text{proteinate}]^- + [HCO_3]^- + [Cl]^- + [(HPO_4)^{--} + (H_2PO_4)^-] + [SO_4]^{--}$. On the other hand, the total base equals $[Na]^+ + [K]^+ + [Ca]^{++} + [Mg]^{++}$, which represents the sum of the cations.

Cell volumes were determined by using Plass and Rourke sedimentation tubes† and rotating them at 3,000 r.p.m. for thirty minutes.

Oxygen and carbon dioxide contents and capacities were determined by the manometric method of Van Slyke and Neill,[8] using the gas apparatus and technique of Van Slyke.[9] Factors of 0.1 and 0.5 volume per cent were subtracted from the oxygen content and capacity, respectively, for the dissolved and the free oxygen. If we assume that one molecule of oxygen combines with one molecule of hemoglobin, the corrected oxygen capacity divided by 2.24 gives the millimols of hemoglobin.

The hydrogen-ion concentration of the plasma was determined by a K-type potentiometer with a quinhydrone microelectrode similar to that described by Cullen.[10]

Volumes per cent of carbon dioxide were converted into milliequivalents present as bicarbonate by the following equation[11, 12]:

$$[BHCO_3] = [CO_2] - \frac{[CO_2]}{10^{pH - pK^1} + 1}$$

in which $[CO_2]$ represents the millimolar concentration of total CO_2 or $\frac{\text{Volume per cent } CO_2 \times 0.01976}{0.044}$. pK^1 values of 6.10 and 6.13 were used for plasma and whole blood, respectively. The factor $\frac{[CO_2]}{10^{pHs - 6.10} + 1}$ represents the concentration of free carbonic acid in the plasma.

The alkali reserve was calculated from the volume per cent carbon-dioxide combining power by means of the formula used to determine the amount of $BHCO_3$.

Carbon dioxide tension was calculated from the carbonic acid concentration in the plasma by the following formula:

$$p - CO_2 \text{ mm.} = \frac{[H_2CO_3]_s}{0.0327}.$$

*A milliequivalent, or millimol (mEq. or mM), of a given constituent represents $\frac{1}{1000}$ part of its hydrogen equivalent or of its molecular weight, respectively, per kilogram of water. For example, 1.0 mEq. of sodium equals $\frac{1}{1000}$ of its atomic weight divided by 1.008 (its hydrogen equivalent), or $\frac{23}{1,000} \div 1.008 = 0.023$.

†Manufactured by Macalaster, Bicknell Co., 40 Wendell St., Cambridge, Mass.

The Folin-Wu method[13] was used to determine the nonprotein nitrogen in plasma and whole blood.

The total nitrogen of plasma was determined by the micro-Kjeldahl method.[14] After the nonprotein nitrogen was subtracted, the protein nitrogen was multiplied by the factor 6.25, to obtain the total protein percentage, which was converted into milliequivalents of base combined with protein, [BP], by the formula of Peters, Wakeman, Eisenman, and Lee,[15] [BP] = 1.072 P (pH – 5.04), in which an albumin-globulin ratio of 1.8 was assumed.

The Wilson and Ball procedure for chlorides in whole blood and plasma was employed.[16] The milligrams per cent of sodium chloride divided by 5.85 represent the milliequivalents of chlorides in 1,000 c.c.

Inorganic phosphorus was determined by the method of Youngburg and Youngburg.[17] The milligrams per cent of phosphorus was reduced to milliequivalents of $[HPO_4]^{--} + [H_2PO_4]^-$ per liter by the factor $\frac{18}{31.04}$, accepting Henderson's[18] estimate of the proportion of primary and secondary phosphates.

In the sulfate determinations the benzidine sulfate was prepared according to Wakefield,[19] but instead of proceeding to a colorimetric comparison, a titrimetric method was employed, using 0.02 normal sodium hydroxide with phenol red as the indicator. Assuming that each atom of sulfur combines with two equivalents of monovalent base, 1 mg. per cent of sulfate is equivalent to 0.208 mEq. of combined base.

The specific gravities were determined by weighing 2.0 c.c. of plasma or whole blood in a 25 c.c. silica crucible. The water content was obtained by drying the specimen at 105° C. to constant weight.

Potassium was determined on the dried material remaining in the crucible, which was treated with one or two drops of concentrated sulfuric acid and ashed overnight at a low heat in an electric muffle. The ash was dissolved in a few drops of dilute hydrochloric acid and water. The solution obtained from the plasma specimen was transferred to a 15 c.c. centrifuge tube and evaporated to dryness, while that from the whole blood was transferred to a 15 c.c. graduated centrifuge tube, made up to 10 c.c. with water, and centrifuged to separate the insoluble iron oxide; an aliquot portion of the clear supernatant solution was transferred to another centrifuge tube and evaporated to dryness. The residues from the serum and whole blood were analyzed for potassium by the method of Shohl and Bennett.[20] One milligram per cent of potassium is equivalent to $\frac{1}{3.91}$, or 0.256 mEq. per liter.

The serum sodium was determined by the zinc uranyl acetate technique of Salit.[21] The direct method previously described by one of us[22] was used to determine the cell sodium, the concentration values of which are recorded. One milligram per cent of sodium is equivalent to $\frac{1}{23}$, or 0.0434 mEq. per liter.

Calcium was determined by the Clark and Collip[23] modification of the Kramer-Tisdall procedure.[24] One milligram per cent of calcium is equivalent to $2 \times \frac{1}{4.0}$, or 0.5 mEq. per liter.

TABLE I

ACID-BASE EQUILIBRIUM IN PLASMA OF NORMAL NONPREGNANT, LATE PREGNANT, AND PUERPERAL WOMEN

(Concentrations of constituents are reported in terms of milliequivalents per kilogram of water unless otherwise stated.)

MATERIAL	NONPREGNANT		LATE PREGNANT PRIMIGRAVIDAS		LATE PREGNANT MULTIGRAVIDAS		POST PARTUM (7-9 DAYS)	
	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE
No. of cases	10		10		10		10	
pH	7.39	7.33- 7.47	7.37	7.30- 7.48	7.40	7.33- 7.48	7.39	7.32- 7.43
$[Cl]^-$	111.3	106.5 -115.9	111.3	103.1 -114.5	113.2	104.9 -121.8	112.0	107.3 -117.0
$[HCO_3]^-$	27.4	24.3 - 30.9	24.9	20.3 - 27.8	24.2	21.6 - 27.3	28.1	26.4 - 29.4
$[Proteinate]^-$	18.4	15.6 - 21.7	15.9	13.5 - 20.1	15.6	13.6 - 20.3	18.4	16.5 - 21.1
$[HPO_4]^{--} + [H_2PO_4]^-$	2.5	2.3 - 2.8	2.4	2.1 - 2.7	2.3	2.0 - 2.7	2.5	2.2 - 2.9
$[SO_4]^{--}$	0.5	0.2 - 1.0	0.6	0.3 - 1.2	0.5	0.2 - 1.2	0.7	0.2 - 1.3
Total acids	160.1	153.5 -168.5	155.1	147.4 -161.2	155.8	148.6 -162.8	161.7	155.4 -166.4
$[Na]^+$	156.7	151.1 -164.7	151.9	150.7 -157.5	151.3	140.8 -162.7	158.0	151.2 -162.6
$[K]^+$	4.8	4.2 - 6.0	5.0	4.1 - 6.0	5.2	4.1 - 6.1	4.7	4.2 - 5.3
$[Ca]^{++}$	5.5	4.6 - 6.4	5.2	4.8 - 6.0	5.1	4.7 - 5.9	5.8	4.6 - 6.4
$[Mg]^{++}$	2.4	2.0 - 2.8	2.3	1.9 - 2.9	2.4	1.9 - 2.9	2.3	1.9 - 3.2
Total bases	169.2	161.0 -176.7	164.2	161.6 -169.1	164.0	152.9 -176.0	170.8	162.9 -176.6
p-CO_2 mm.	43.7	32.1 - 55.5	41.9	31.2 - 50.6	37.3	31.7 - 44.7	45.2	41.1 - 54.0
H_2CO_3 mM	1.43	1.05- 1.81	1.37	1.02- 1.65	1.22	1.04- 1.46	1.48	1.34- 1.76
N.P.N. mg. per cent	30.3	25.5 - 36.5	31.6	25.0 - 40.4	31.9	25.0 - 44.4	38.1	28.2 - 44.2
Alkali reserve	31.5		28.7		27.7		30.6	

TABLE II

ACID-BASE EQUILIBRIUM IN CELLS OF NORMAL NONPREGNANT, LATE PREGNANT, AND PUERPERAL WOMEN

(Concentrations of constituents are reported in terms of milliequivalents per kilogram of water unless otherwise stated.)

MATERIAL	NONPREGNANT		LATE PREGNANT PRIMIGRAVIDAS		LATE PREGNANT MULTIGRAVIDAS		POST PARTUM (7-9 DAYS)	
	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE
$[Cl]^-$	79.2	72.4 - 86.9	74.9	59.2 - 87.8	71.7	45.2 - 87.1	72.6	57.8 - 85.0
$[HCO_3]^-$	24.0	18.7 - 26.6	20.7	16.0 - 23.9	19.5	14.1 - 22.1	23.1	20.2 - 25.7
$[Proteinate]^{-}$	45.3		45.5		49.2		48.4	
$[HPO_4]^{--} + [H_2PO_4]^-$	3.2	2.6 - 4.5	3.2	2.1 - 4.5	3.3	2.0 - 5.9	3.6	2.9 - 4.8
Total acids	151.7		144.5		143.7		147.7	
$[K]^+$	136.2	127.2 -150.6	138.8	129.2 -149.1	139.7	122.0 -164.3	146.9	131.1 -150.2
$[Na]^+$			16.6		16.6			
O_2 capacity volume per cent	16.79		15.95		15.35		18.94	
O_2 content volume per cent	7.62		7.73		8.22		9.71	
pH estimated	7.17		7.15		7.18		7.17	
Alkali reserve	24.4		26.2		25.1		23.0	

The method of Denis[25] was employed for the determination of magnesium. One milligram per cent of magnesium is equivalent to $2 \times \frac{1}{2.43}$, or 0.819 mEq. per liter.

The base bound by hemoglobin, or base protein, $[BP]_c$, was calculated according to the equation of Van Slyke, Wu, and McLean:[26]

$$[BP]_c = 3.35\ [Hb]_c\ (pH_c - 6.74) + [O_2]_c\ (0.25\ pH_c - 1.18)$$

where $[Hb]_c$ represents millimols of hemoglobin per kilogram of water; $[O_2]_c$, the millimols of oxygen per kilogram of water, and pH_c, the hydrogen-ion concentration within the cells as estimated from a graph using plasma pH data.[26] The values thus obtained may not represent the base actually bound by the hemoglobin in human blood, since the equation was derived from data on horse hemoglobin, but they undoubtedly have some value for comparative purposes.

RESULTS

The average values for the various constituents (Table I), except those for the pH, are in accord with the reports of previous investigators.[1-6] The average pH value is the same in the pregnant women as in the nonpregnant, thus confirming the observations of Kydd, Oard, and Peters,[4] and others. The total acid and the total base are diminished approximately 5.5 mEq. during late pregnancy but return to the nonpregnant level within seven to nine days after delivery. Reductions of the bicarbonate and of the proteinate ions account chiefly for the decrease in total acid, while the lowered sodium explains the reduced total base, which is associated with a lowered alkali reserve. The average values for carbon dioxide tension and for carbonic acid, as calculated from the total carbon dioxide content of the plasma, are slightly reduced in late pregnancy but return to normal shortly after delivery.

Since all values recorded for the blood cells (Table II) have been calculated from the analyses of plasma or serum and of whole blood, and from hematocrit determinations, it is obvious that they are subject to undue variations as the result of a possible summation of analytic errors. Average values for cell chloride and bicarbonate in the nonpregnant group agree with those reported by Hastings, Sendroy, McIntosh, and Van Slyke,[12] but they are lower in the groups of women in late pregnancy; this confirms the observations of Muntwyler, Myers, and Way.[28] The cell proteinates and phosphates show no significant variations in the different groups. During pregnancy the total acids in the cells are approximately 7.6 mEq. per liter below the nonpregnant level, and by the seventh to the ninth days post partum they have only partially returned to normal, being still 4.0 mEq. under the nonpregnant average.

The cell potassium, which represents most of the total base in the cells, is slightly elevated in the pregnant groups, the rise being associated with an increased hemoglobin content per kilogram of water, as previously reported.[7] During the early puerperium the cell potassium rises still higher, and there is no indication as to when it returns to normal. Values for the sodium content of the cells are available only for the pregnant groups, and consequently no comparison can be attempted. The alkali reserve is increased slightly in the

cells during gestation but is decreased in the plasma. After delivery both serum potassium and plasma alkali reserve return promptly to the normal nonpregnant level, in spite of the persistent elevation of the cell potassium.

DISCUSSION

The recorded pregnancy changes in the various plasma constituents confirm the observations of previous investigators and require no comment.

During pregnancy the diffusible constituents of the blood cells vary generally with the alterations in the plasma to maintain a Donnan type of equilibrium. The total base of the cells is not influenced by the reduction in the total base of the plasma but actually rises slightly because it consists largely of nondiffusible potassium, which is closely associated with the equally fixed hemoglobin. This situation probably explains the increased alkali reserve of the cells. The amount of base bound with hemoglobin to form a proteinate is governed by the pH and the carbon dioxide content of the blood. In general, the variations in the acid-base balance of the cells are due directly to alterations in the concentrations of the diffusible constituents of the plasma.

An interpretation of the acid-base balance in pregnant and puerperal women has been presented in a previous communication dealing with pulmonary ventilation.[29]

SUMMARY

1. A detailed study of the acid-base equilibrium of the plasma and cells in normal ten nonpregnant, twenty pregnant, and ten puerperal women is reported.

2. The reduction of the total acid constituents of the plasma, which occurs during pregnancy, is balanced by a corresponding reduction in the total base, with both factors returning to normal early in the puerperium.

3. The pH of the plasma remains at the normal nonpregnant level during pregnancy and early puerperium.

4. The free carbonic acid and the carbon dioxide tension of the plasma fall slightly in late pregnancy but return to the nonpregnant level shortly after delivery.

5. The total acid constituents in the cells are slightly diminished in late pregnancy but return to normal within nine days after delivery.

6. The potassium concentration in the cells, which is increased in late pregnancy, shows a further rise during the early puerperium.

7. The alkali reserve of the cells is increased during pregnancy but falls to the nonpregnant level within a few days after confinement.

REFERENCES

1. Oard, H. C., and Peters, J. P.: The Concentration of Acid and Base in the Serum in Normal Pregnancy, J. Biol. Chem. **81:** 9, 1929.
2. Muntwyler, E., Limbach, N., Bill, A. H., and Myers, V. C.: Acid-Base Equilibrium of Blood in Pathological Conditions; Changes Observed in Toxemias of Pregnancy, J. Biol. Chem. **90:** 607, 1931.
3. Kydd, D. M.: Hydrogen-Ion Concentration and Acid-Base Equilibrium in Normal Pregnancy, J. Biol. Chem. **91:** 63, 1931.

4. Kydd, D. M., Oard, H. C., and Peters, J. P.: The Acid-Base Equilibrium in Abnormal Pregnancy, J. Biol. Chem. **98:** 241, 1932.
5. Myers, V. C., Muntwyler, E., and Bill, A. H.: The Acid-Base Balance Disturbance of Pregnancy, J. Biol. Chem. **98:** 253, 1932.
6. Nice, M., Mull, J. W., Muntwyler, E., and Myers, V. C.: The Acid-Base Balance of the Blood During Normal Pregnancy and Puerperium, Am. J. Obst. & Gynec. **32:** 375, 1936.
7. Oberst, F. W., and Plass, E. D.: Water Concentration of the Blood During Pregnancy, Labor, and the Puerperium, Am. J. Obst. & Gynec. **31:** 61, 1936.
8. Van Slyke, D. D., and Neill, J. M.: Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement, J. Biol. Chem. **61:** 523, 1924.
9. Van Slyke, D. D.: Note on a Portable Form of the Manometric Gas Apparatus and on Certain Points in the Technique of Its Use, J. Biol. Chem. **73:** 121, 1927.
10. Cullen, G. E.: Improved Form of Quinhydrone Electrode, J. Biol. Chem. **83:** 535, 1929.
11. Austin, J. H., Cullen, G. E., Hastings, A. B., McLean, F. C., Peters, J. P., and Van Slyke, D. D.: Studies of Gas and Electrolyte Equilibria in Blood. I. Technique for Collection and Analysis of Blood, and for Its Saturation With Gas Mixtures of Known Concentration, J. Biol. Chem. **54:** 121, 1922.
12. Hastings, A. B., Sendroy, J., Jr., McIntosh, J. F., and Van Slyke, D. D.: Studies of Gas and Electrolyte Equilibria. XIII. The Distribution of Chloride and Bicarbonate in the Blood of Normal and Pathological Human Subjects, J. Biol. Chem. **79:** 193, 1928.
13. Folin, O., and Wu, H.: A System of Blood Analysis, J. Biol. Chem. **38:** 81, 1919.
14. Hawk, P. B., and Bergeim, O.: Practical Physiological Chemistry, ed. 10, Philadelphia, 1931, P. Blakiston's Son & Co., p. 449.
15. Peters, J. P., Wakeman, A. M., Eisenman, A. J., and Lee, C.: Total Acid-Base Equilibrium of Plasma in Health and Disease. X. The Acidosis of Nephritis, J. Clin. Investigation **6:** 517, 1929.
16. Wilson, D. W., and Ball, E. G.: A Study of the Estimation of Chloride in Blood and Serum, J. Biol. Chem. **79:** 221, 1928.
17. Youngburg, G. E., and Youngburg, M. V.: Phosphorus Metabolism. I. A System of Blood Phosphorus Analysis, J. LAB. & CLIN. MED. **16:** 158, 1930.
18. Henderson, L. J.: The Theory of Neutrality Regulation in the Animal Organism, Am. J. Physiol. **21:** 427, 1908.
19. Wakefield, E. G.: Colorimetric Determination of Total Inorganic Sulfates in Blood Serum, Urine, and Other Body Fluids, J. Biol. Chem. **81:** 713, 1929.
20. Shohl, A. T., and Bennett, H. B.: A Micro Method for the Determination of Potassium as Iodoplatinate, J. Biol. Chem. **78:** 643, 1928.
21. Salit, P. W.: A New Triple Acetate Method for Sodium Determinations in Biological Materials, J. Biol. Chem. **96:** 659, 1932.
22. Oberst, F. W.: The Determination of Sodium in Human Red Blood Cells, J. Biol. Chem. **108:** 153, 1935.
23. Clark, E. P., and Collip, J. B.: A Study of the Tisdall Method for the Determination of Blood Serum Calcium, With a Suggested Modification, J. Biol. Chem. **63:** 461, 1925.
24. Kramer, B., and Tisdall, F. F.: A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum, J. Biol. Chem. **47:** 475, 1921.
25. Denis, W.: The Determination of Magnesium in Blood, Plasma, and Serum, J. Biol. Chem. **52:** 411, 1922.
26. Van Slyke, D. D., Wu, H., and McLean, F. C.: Studies of Gas and Electrolyte Equilibria in Blood; Factors Controlling Electrolyte and Water Distribution in Blood, J. Biol. Chem. **56:** 765, 1923.
27. Taylor, H.: The Hydrogen-Ion Concentration of Blood Corpuscles, J. Physiol. **63:** 343, 1927.
28. Muntwyler, E., Myers, V. C., and Way, C. T.: The Distribution of Chloride and Bicarbonate Between Plasma and Cells in the Blood of Various Pathological Conditions, J. Biol. Chem. **92:** 721, 1931.
29. Plass, E. D., and Oberst, F. W.: Respiration and Pulmonary Ventilation in Normal Nonpregnant, Pregnant, and Puerperal Women, Am. J. Obst. & Gynec. **35:** 441, 1938.

THE MAGNESIUM CONTENT OF THE BLOOD SERUM AND URINE*

MITCHELL BERNSTEIN, M.D., F.A.C.P., AND SAMUEL SIMKINS, M.D.
PHILADELPHIA, PA.

BECAUSE of the increasing importance of magnesium in the field of cardiovascular-renal disease and because of the relative dearth of data in the literature, the present investigation of the magnesium content of the blood serum and urine was undertaken.

The patients studied comprised 201 consecutive medical ward cases, divided almost equally between both sexes. A total of 325 blood serum determinations was performed. Insofar as could be determined the patients had no disturbance of mineral metabolism. In general, a fasting blood magnesium test was made although it has been shown that no noticeable difference exists in the magnesium content of the blood before and after meals.[1] The method employed for the determination of magnesium was the modification of the Titan yellow dye method recently perfected by Haury,[2] a method so essentially simple and dependable that it ought to mark a great step forward in the clinical investigation of magnesium metabolism.

The statement is seen repeatedly in the literature that the amount of blood magnesium is remarkably constant, varies within very narrow limits only, and is practically uninfluenced by pathologic processes.[3, 4] Yet a careful perusal of the literature disclosed very marked divergences in the recorded data.

The patients were divided into a noncardiac group with a wide miscellany of diseases, a cardiac group, and a nephritic group. The combined cardiac and noncardiac group totaled 197 patients in whom 312 blood serum magnesium tests were performed. The average magnesium value was 2.1 mg. per cent which agreed fairly well with the results of Becher[5] (1.8 to 2.3), Bomskov[6] (1.7 to 2.6), Velluz and Velluz[7] (2.00) (1.62 to 2.40), Brookfield[8] (2.04) (1.89 to 2.19), Cope[9] (2.06) (1.82 to 2.63), and Walker and Walker[10] (1.5 to 2.9), but was considerably below the values obtained by Greenberg and others[11] (2.74) (2.0 to 3.6), Briggs[12] (2.23 to 2.50), Wacker and Fahrig[13] (2.4) (2.03 to 2.97), Denis[14] (1.6 to 3.5), Cohen[15] (2.56) (2.09 to 2.74), Marriott and Howland[16] (2.2 to 3.5), Kramer and Tisdall[17] (2.3 to 4.0), Watchorn and McCance[18] (2.3 to 2.65), Watchorn[19] (2.2 to 2.6), and McCance and Watchorn[20] (2.00 to 4.05). A noteworthy feature is the marked individual variations we found (1.23 to 3.54) in support of the findings of Greenberg and co-workers.[11] Evidence of the serum magnesium does not vary within such narrow limits as was hitherto supposed.

*From the Medical Service of Dr. Mitchell Bernstein, Jewish Hospital, Philadelphia.
Read before the Jefferson Society for Clinical Investigation, Jefferson Medical College, Philadelphia, December 21, 1939.
Received for publication, November 7, 1939.

NONCARDIAC GROUP

The noncardiac group included 94 patients in whom 138 magnesium tests were performed. The average serum magnesium was 2.12, with extremes of 1.37 to 3.11. A division of this group into smaller groups of patients with definite diseases (such as pneumonia, irritable colon, cholelithiasis, bronchial asthma) disclosed no great variations from the general average. The pregnancy group (comprising 4 cases) tended to show somewhat lower values in support of the findings of Watchorn and McCance.[18] The miscellaneous section comprised a great variety of diseases, including erysipelas, Hodgkin's disease, scleroderma, Henoch's purpura, bronchiectasis, psoriasis, thromboangiitis obliterans, ulcerative colitis, and chronic lymphatic leucemia.

TABLE I

BLOOD SERUM MAGNESIUM VALUES

DIAGNOSIS	NO. OF PATIENTS	NO. OF TESTS	AVG. BLOOD SERUM (MG. %)	BLOOD SERUM (MG. %) (EXTREMES)
A. *Noncardiac Group*				
Pneumonia	12	16	1.96	1.23-2.86
Cholelithiasis	9	17	2.02	1.38-2.60
Irritable colon	8	11	2.14	1.69-2.83
Pyelitis and nephroptosis	6	8	1.96	1.58-2.40
Bronchial asthma	5	10	2.12	1.66-2.91
Diabetes mellitus	4	7	2.06	1.62-2.58
Pregnancy	4	4	1.99	1.68-2.30
Duodenal ulcer	3	4	2.28	2.04-2.44
Miscellaneous	43	61	2.21	1.37-3.11
Noncardiac total	94	138	2.12	1.37-3.11
B. *Cardiac Group*				
Ambulatory hypertensives	23	34	2.25	1.67-3.31
Ward hypertensives	31	57	2.27	1.63-3.41
Arteriosclerotic heart disease	35	66	2.23	1.57-3.54
Miscellaneous heart disease	14	17	2.30	1.29-3.20
Cardiac total	103	174	2.25	1.29-3.54
Total groups A and B	197	312	2.19	1.23-3.54
C. *Chronic Nephritis*				
Chronic nephritis	4	13	3.31	1.89-4.60

CARDIAC GROUP

The cardiac group numbered 103, of whom 54 had hypertensive heart disease. The magnesium level in this condition is still a mooted question. Wacker and Fahrig[13] state that magnesium is the only mineral found in increased concentration in the serum in essential hypertension. Their average magnesium value was 2.41 as compared with their normal of 2.28. In a group of 15 hypertensive patients without severe renal damage Walker and Walker[10] found an average value of 2.36, as compared with their normal value of 2.2 in normal active young adults, and 2.3 in miscellaneous medical and surgical hospital cases. In 1923 Weil, Guillaumin, and Weismann-Netter[21] found an increased serum magnesium in a few cases of essential hypertension, but to quote Walker and Walker:[10] "These figures deviate so far, however, from the standards used by other authors that comparison is not possible." In our series were 23 ambulatory

hypertensive patients whose magnesium levels were 2.5 (1.67 to 3.31) and 31 ward hypertensive patients of whom many were decompensated with values of 2.27 (1.63 to 3.41). The grand average for the hypertensive group was 2.26, as compared with our normals of 2.12. The increase is very slight but is in conformity with the results obtained by the authors quoted above. Whether any significance is to be attached to so small a difference is certainly open to question.

The arteriosclerotic heart disease group numbered 35 patients in whom 66 magnesium determinations were effected. The average value was 2.23 mg. per cent, with extremes of 1.57 to 3.54. In the miscellaneous heart disease group were 14 patients in whom 17 blood magnesium studies were made. The group was heterogenous and included 4 cases of chronic rheumatic heart disease, 2 of congenital heart disease, one of acute rheumatic fever, 3 of subacute bacterial endocarditis, one of syphilitic heart disease, one of scarlatinal heart block,[31] and 2 of thyrotoxic heart disease. The average magnesium value for the foregoing group was 2.24 (1.29 to 3.30). It is noteworthy that the magnesium value for nonhypertensive patients with cardiac disorders is elevated to practically the same degree as in patients in the hypertensive group, though again emphasis must be laid on the fact that the increase is very slight (Table I).

NEPHRITIC GROUP

The question of the magnesium content of the serum in renal insufficiency is still in a state of flux. One group affirms that there is no retention of magnesium in renal insufficiency. In this group are Denis and Hobson,[22] who obtained figures of 2.2 to 2.9 in 19 patients, Denis,[23] working in experimental nephritis, and Fishberg.[24] In 1923 Salvesen and Linder[25] reported 15 patients in whom they sometimes found a reduction and sometimes an increase in magnesium (1.5 to 4.6). Somewhat similar results were obtained a year later by Rabinowitch[26] (1.6 to 3.1). That magnesium is retained in renal insufficiency has been asserted by another group of investigators. Included are Becher,[5] Becher and Hamann,[27] who found values of 2.7 to 5.7 in 10 cases; Walker and Walker,[10] who found abnormally high serum magnesium values in 5 hypertensive persons with renal damage (2.07 to 4.20); Cope,[9] who in 1936 found the serum magnesium considerably elevated in 5 patients with renal damage increased after intensive alkali administration (4 patients with readings over 4.00 and one with a reading of 6.84); Hirschfelder,[28, 29] who in 1934 reported serum magnesium figures often considerably raised in renal inadequacy; and Brookfield,[8] who confirmed the increase of serum magnesium in renal dysfunction in a series of 21 cases (values did not exceed 3.93).

In the present investigation, 4 cases of nephritis were studied. The average reading vas 3.31 (1.89 to 4.60).

CASE 1.—L. F., a female, aged 24 years, had had chronic nephritis of a mild character for several years but no renal insufficiency. Blood magnesium values taken several weeks apart were 1.89 and 2.55.

CASE 2.—R. C., a male, aged 63 years, had hypertension with definite chronic glomerulonephritis but only slight renal insufficiency. Blood magnesium determinations within a period of two weeks were 2.90, 2.86, and 2.06 keeping pace with the patient's clinical improvement.

CASE 3.—C. B., a female, aged 17 years, had advanced chronic glomerulonephritis. Blood magnesium was 4.36 on admission, 4.17 on the sixth day, 3.99 on the seventh, and 4.36 on the thirteenth. She received 1 ounce of magnesium sulfate by mouth on the sixth and seventh days, and 1.5 ounces on the ninth, tenth, eleventh, and thirteenth days, as well as an intramuscular injection of 2 c.c. of 50 per cent magnesium sulfate daily from the eighth to thirteenth day inclusive.

CASE 4.—M. J., a hypertensive negress, aged 38 years, had chronic glomerulonephritis with extreme degree of renal insufficiency. Initial blood magnesium on admission to the hospital (July 16, 1937) was 2.8. She received intramuscular injections of 2 c.c. of 50 per cent magnesium sulfate twice daily from August 3 to August 16 inclusive. Blood magnesium level on August 5 was 2.90, on August 12, 3.68, and on August 16, 4.60. The patient became comatose on August 19 and died the following day. Post-mortem examination confirmed the presence of an advanced glomerulonephritis. The results obtained bear out Brookfield's[8] findings that an increase of serum magnesium is almost invariably present in renal dysfunction and corroborate his failure to disclose magnesium levels sufficient to produce coma as advanced by Hirschfelder.[29]

URINARY MAGNESIUM

There are very few recorded experimental studies covering the level of the urinary magnesium. Cope[9] recorded the basic urinary output of magnesium in one patient as 50 mg. per day. Walker and Walker[10] found the twenty-four-hour urinary output in a group of normal active young adults to average 103 mg. (32.5 to 307.0 mg.) and 86 mg. (5 to 243 mg.) in a group of miscellaneous medical and surgical hospital patients. In 8 young adults Weber[30] recently reported the average daily urinary excretion of magnesium to be 83.9 to 132.1 mg. In our series the average daily excretion of 57 patients on admission to the hospital was found to be 105.5 mg., with values ranging from 17.3 to 285.0 mg. The values agree closely with those obtained by Walker and Walker. Such high variations as we obtained might readily be expected a priori when the great range of the individual diet is considered.

In several patients intramuscular injections of magnesium sulfate were given with a consequent reflection in the urinary output. In a hypertensive patient the initial urinary output was 45.3 mg. After 2 c.c. of 50 per cent magnesium sulfate were injected intramuscularly twice daily for four days, the urinary output rose to 102.9 and to 140.7 mg. the subsequent day.

In a patient with arteriosclerotic heart disease the initial urinary value was 80.0, rose to 158 the day following the intravenous injection of 15 c.c. of 10 per cent magnesium sulfate, reached 298.0 following the injection of 2 c.c. of 50 per cent magnesium sulfate intramuscularly twice daily for four days, and rose to a peak of 416.1 after the intramuscular injections were continued for eleven days more. No correlation could be established between the urine output and the blood level.

CONCLUSIONS

1. A total of 325 determinations of the blood serum magnesium were made in 201 medical cases with no disturbance of mineral metabolism.

2. A total of 312 determinations in 197 of the patients (excluding the 4 nephritic patients in the series) showed an average value of 2.19 mg. per cent (1.23 to 3.54).

(a) A total of 138 magnesium tests in 94 noncardiac patients showed an average value of 2.12 per cent (1.37 to 3.11).

(b) Ninety-one magnesium tests in 54 hypertensive persons gave an average value of 2.26 (1.63 to 3.41).

(c) Sixty-six magnesium determinations in 35 arteriosclerotic heart disease patients showed an average value of 2.23 (1.57 to 3.54).

(d) Seventeen magnesium tests in 14 patients with various other forms of heart disease showed an average magnesium value of 2.24 (1.29 to 3.30).

3. In 4 cases of chronic nephritis with varying degrees of renal insufficiency the blood serum magnesium level was elevated to almost double the normal value (3.31) (1.89 to 4.60).

4. The average twenty-four-hour urinary output of magnesium in 57 persons was 105.5 mg. (17.3 to 285.0).

1321 SPRUCE STREET

REFERENCES

1. Watchorn, E.: Thesis, Cambridge, 1929.
2. Haury, V. G.: Modification of Titan Yellow Method for Determination of Small Amounts of Magnesium in Biological Fluids, J. LAB. & CLIN. MED. **23**: 1079, 1938.
3. Sholl, A. T.: Mineral Metabolism—Calcium and Magnesium, Ann. Rev. Biochem. **2**: 207, 1933.
4. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, 1932, Williams & Wilkins Co., Vol. 1.
5. Becher, E.: Der Magnesiumgehalt des Blutes unter normalen und pathologischen Verhaltnissen, insbesondere bei Nierenkrankheiten, Klin. Wchnschr. **11**: 202, 1932.
6. Bomskov, C.: Determination of Magnesium in Blood With 8-hydroxy quinoline, J. Biol. Chem. **99**: 17, 1932.
7. Velluz, L., and Velluz, J.: Sur les variations physiologiques du magnesium sériques, Compt. rend. Soc. de biol. **117**: 417, 1934.
8. Brookfield, R. W.: Magnesium Content of Serum in Renal Insufficiency, Quart. J. Med. **6**: 87, 1937.
9. Cope, C. L.: Base Changes in the Alkalosis Produced by the Treatment of Gastric Ulcer With Alkalies, Clin. Sc. **2**: 287, 1936.
10. Walker, B. S., and Walker, E. W.: Normal Magnesium Metabolism and Its Significant Disturbances, J. LAB. & CLIN. MED. **21**: 713, 1936.
11. Greenberg, D. M., Lucia, S. P., Mackey, M. A., and Tufts, E. V.: Magnesium Content of the Plasma and Red Corpuscles in Human Blood, J. Biol. Chem. **100**: 139, 1933.
12. Briggs, A. P.: A Colorimetric Method for the Determination of Small Amount of Magnesium, J. Biol. Chem. **52**: 349, 1922.
13. Wacker, L., and Fahrig, C.: Über die mineralischen und lipoiden Bestondterle des Blutserums bei der essentiellen Hypertension un Vergleich zu den physiologischen Verhaltnissen, Klin. Wchnschr. **11**: 762, 1932.
14. Denis, W.: Determination of Magnesium in Blood, J. Biol. Chem. **41**: 363, 1920.
15. Cohen, H.: Magnesium Content of the Cerebrospinal and Other Body Fluids, Quart. J. Med. **20**: 173, 1927.
16. Marriott, W. McK., and Howland, J.: A Micro-method for the Determination of Calcium and Magnesium in Blood Serum, J. Biol. Chem. **32**: 233, 1917.
17. Kramer, B., and Tisdall, F. T.: The Distribution of Sodium, Potassium, Calcium and Magnesium Between the Corpuscles and Serum of Human Blood, J. Biol. Chem. **53**: 241, 1922.
18. Watchorn, E., and McCance, R. A.: Inorganic Constituents of Cerebrospinal Fluid. II. The Ultrafiltration of Calcium and Magnesium From Human Sera, Biochem. J. **26**: 54, 1932.
19. Watchorn, E.: Calcium and Magnesium in Some Pathological Sera, Quart. J. Med. **18**: 288, 1925.
20. McCance, R. A., and Watchorn, E.: Inorganic Constituents of Cerebrospinal Fluid. I. Calcium and Magnesium, Quart. J. Med. **24**: 371, 1931.
21. Weil, M., Guillaumin, C., and Weismann-Netter, R.: Le calcium et le magnesium seriques chez l'adulte normal, hypertendu on atheromateux, Compt. rend. Soc. de biol. **88**: 732, 1923.
22. Denis, W., and Hobson, S.: A Study of the Inorganic Constituents of the Blood Serum in Nephritis, J. Biol. Chem. **55**: 183, 1923.
23. Denis, W.: Study of the Inorganic Constituents of the Blood in Experimental Nephritis, J. Biol. Chem. **56**: 473, 1923.

24. Fishberg, A. M.: Hypertension and Nephritis, ed. 3, Philadelphia, 1934, Lea and Febiger, p. 29.
25. Salvesen, H. A., and Linder, G. C.: Observations on the Inorganic Bases and Phosphates in Relation to the Problem of Blood and Other Body Fluids in Bright's Disease and in Heart Failure, J. Biol. Chem. **58**: 617, 1923.
26. Rabinowitch, I. M.: On the Relative Proportions of Sodium, Potassium, Calcium and Magnesium in Blood Plasma in Renal Disease, J. Biol. Chem. **52**: 667, 1925.
27. Becher, E., and Hamann, K.: Studien über das Verhalten des Magnesium im Organismus insbesondere in Blut, Deutsches Arch. f. klin. Med. **173**: 500, 1932.
28. Hirschfelder, H. D.: Effect of Renal Insufficiency Upon Plasma Magnesium and Magnesium Excretion After Ingestion of Magnesium Sulfate, J. Biol. Chem. **104**: 647, 1934.
29. Hirschfelder, H. D.: Clinical Manifestations of High and Low Plasma Magnesium. Dangers of Epsom Salt Purgation in Nephritis, J. A. M. A. **102**: 1138, 1934.
30. Weber, R. J.: Excretion of Some Purgative Salts of Magnesium, Proc. Soc. Exper. Biol. & Med. **37**: 55, 1937.
31. Bernstein, Mitchell: Auriculoventricular Dissociation Following Scarlet Fever, Am. Heart J. **16**: 582, 1938.

INFLUENCE OF GLYCEROL ON GLYCEMIA IN NORMAL AND DIABETIC INDIVIDUALS*

MAX WISHNOFSKY, M.D., ARTHUR P. KANE, M.D., WILLIAM C. SPITZ, M.D., SAUL MICHALOVER, M.D., AND CHARLES S. BYRON, M.D.
BROOKLYN, N. Y.

THE relationship of substances other than hexoses, especially those having three carbon atoms, to carbohydrate metabolism has received considerable attention. Our knowledge of the metabolism of some of these substances is, however, in many respects fragmentary. In this paper we shall concern ourselves with the metabolism of glycerol. Although much work has been done on this subject, there are certain important phases which have received only scant attention. Experimental work tending to elucidate these problems will be reported.

REVIEW OF LITERATURE

The metabolism of glycerol may be considered from the following standpoints: (1) glycogenesis, (2) antiketogenesis and ketolysis, (3) influence on hypoglycemic shock, (4) influence on blood sugar.

1. Glycogenesis. Catron and Lewis[1] found that the amounts of glycogen formed in the liver after two- and three-hour periods of absorption of glycerol from the gastrointestinal tracts of white rats were equal to those observed after absorption of equivalent amounts of glucose over the same periods. Shapiro[2] has completely confirmed these observations. Chambers and Deuel[3] observed that a practically complete conversion of glycerol into glucose was exhibited by two phlorhizinized dogs. Sansum and Woodyatt[4] found that dl-glyceric aldehyde was quantitatively converted into glucose as determined by measuring glycosuria in deglycogenated phlorhizinized dogs. They argued that this precludes the possibility of glycerol having a greater utilization rate than glucose itself in diabetic persons.

*From the Medical Service of Dr. E. L. Shlevin and the Department of Basal Metabolism of the Jewish Hospital, Brooklyn.
Received for publication, November 9, 1939.

2. Antiketogenesis and ketolysis. Although the antiketogenic action of glycerol was first noted by Hirschfeld in 1895, it was most convincingly demonstrated by Satta's experiments.[2a] Shapiro[2] found that equivalent amounts of glucose and glycerol had equal ketolytic properties.

3. Influence on hypoglycemic shock. Voegtlin and Dunn[5] observed that the symptoms of insulin shock (coma and convulsions) were completely relieved by intravenous or intraperitoneal injections of glycerol. Noble and Macleod,[6] on the contrary, found that glycerol did not affect the symptoms of insulin shock in the slightest degree.

4. Influence on respiratory quotient. The only work on this subject is by McCann and Hannon,[7] who compared the respiratory quotients in three normal persons after the ingestion of glycerol and glucose. They found that whereas a marked rise in respiratory quotient was produced by glucose, no rise occurred with glycerol. It should be mentioned that glucose and glycerol are not strictly comparable, for the respiratory quotient of glucose is 1, whereas that of glycerol is approximately 0.86.

5. Influence on concentration of blood sugar. Voegtlin, Thompson, and Dunn[8] found that glycerol given to fasted rabbits produced a hyperglycemia of considerable extent and duration. This increase was not due to an increase in concentration of the blood. Studies on the influence of glycerol on glycemia in man have been scant. Kneip[9] observed that glycerol given in doses of 0.5 to 0.6 Gm. per kilogram of body weight did not influence the blood sugar of one diabetic and four normal children. Behrens[10] also found that a dose of 30 Gm. of glycerol did not give rise to hyperglycemia in one diabetic and three normal children.

Fats, such as tristearin or triolein, when completely saponified yield approximately ten parts by weight of glycerol to ninety parts by weight of higher fatty acids. As previously mentioned, Sansum and Woodyatt contend that glycerol does not have a greater utilization rate than glucose itself in diabetic persons. It has been customary in estimating the glucose value of a diabetic diet to consider fat as yielding 10 per cent by weight of glucose, and to put that glucose in the same category as that derived from starch.

Investigations on the influence of glycerol on blood sugar have been fragmentary and inadequate. In this paper results of experiments will be reported on the influence of glycerol on blood sugar on a fairly large number of normal and diabetic individuals. The question of the utilization rate of glycerol in diabetic persons will to some extent, for reasons given below, be elucidated by the results obtained.

METHOD OF PROCEDURE

Experiments were performed on 13 normal and 10 diabetic persons. Eighty grams of glycerol,* in sufficient aqueous solution to constitute a total of 500 c.c., were given to each person in the morning after a fast of fourteen hours. Specimens of blood were taken from the normal individuals before, and forty-five minutes, two, and three hours after the ingestion of the glycerol. The sugar

*In the recovery of glucose from glycerol, two molecules of glycerol yield one molecule of glucose and four atoms of hydrogen.
$2C_3H_8O_3 \rightarrow C_6H_{12}O_6 + 4H$, or 80 Gm. of glycerol yield 78.2 Gm. of glucose.

TABLE I

CONCENTRATION OF DEXTROSE IN THE BLOOD OF NORMAL INDIVIDUALS AT THE FASTING LEVEL AND AFTER THE INGESTION OF 80 GM. OF GLYCEROL

CASE NO.	BLOOD SUGAR			
	FASTING LEVEL	FORTY-FIVE MINUTES POSTCIBAL	TWO HOURS POSTCIBAL	THREE HOURS POSTCIBAL
1	106	100	98	
2	86	95	111	
3	97	102	115	
4	103	97	147	117
5	94	104	128	104
6	97	134	126	119
7	95	105	116	118
8	87	80	87	84
9	100	90	111	90
10	92		109	105
11	100	130	147	125
12	98	103	103	98
13	110	112	108	
Means	97	104	116	106

TABLE II

CONCENTRATION OF DEXTROSE IN THE BLOOD OF DIABETIC PERSONS AT THE FASTING LEVEL AND AFTER THE INGESTION OF 80 GM. OF GLYCEROL

CASE NO.	BLOOD SUGAR			
	FASTING LEVEL	ONE HOUR POSTCIBAL	TWO HOURS POSTCIBAL	THREE HOURS POSTCIBAL
1	138	141	145	144
2	171	169	192	198
3	182	225	308	286
4	125	138	135	133
5	166	185	194	173
6	246	282	333	318
7	200	246	254	282
8	194	215	217	210
9	230	286	351	388
10	361	366	400	411
Means	201	225	253	254

TABLE III

CONCENTRATION OF DEXTROSE IN THE BLOOD OF DIABETIC PERSONS AT THE FASTING LEVEL AND AFTER THE INGESTION OF 75 GM. OF GLUCOSE

CASE NO.	BLOOD SUGAR		
	FASTING LEVEL	FORTY-FIVE MINUTES POSTCIBAL	TWO HOURS POSTCIBAL
1	242	400	428
2	250	328	364
3	150	250	300
4	124	364	422
5	176	256	231
6	168	300	378
7	234	356	
8	238	410	374
9	222	378	468
10	98	224	264
11	224	332	364
Means	193	327	359

content of the blood was determined by the Folin-Wu method. In the diabetic patients the blood specimens were taken at the same time with one exception, namely, one hour instead of forty-five minutes after ingestion.

ANALYSIS AND DISCUSSION OF RESULTS

As mentioned in the review of the literature, there has been a paucity of study on the influence of glycerol on glycemia. Voegtlin, Thompson, and Dunn[8] found that glycerol given to fasted rabbits produces a hyperglycemia of considerable extent and duration. Kneip[9] and Behrens,[10] on the other hand, observed that glycerol had no influence on the blood sugar in several normal and diabetic children.

Table I shows the concentrations of the blood sugar in 13 normal individuals at the fasting level and after the ingestion of 80 Gm. of glycerol. A definite rise in the concentration of the blood sugar is noted in 8 of the 13 persons.

Table II shows the concentration of glucose in the blood of 10 diabetic persons at the fasting level and after the ingestion of 80 Gm. of glycerol. In 8 of the 10 cases a definite rise in the concentration of the blood sugar is observed. This rise may be collated with that of a comparable group of diabetic persons who received 75 Gm. of glucose (Table III). It will be noted that the rise in the concentration of the blood sugar when glucose is given is markedly greater than after glycerol.

The question arises: To what extent is this difference the result of a difference in the rates of absorption of the two substances from the human gastrointestinal tract? There has, unfortunately, been no experimental work on this subject. As already mentioned, Catron and Lewis[1] found that the amounts of glycogen formed in the liver after two- and three-hour periods of absorption of glycerol from the gastrointestinal tract of white rats were equal to those observed after the absorption of equivalent amounts of glucose over the same periods. This would indirectly indicate that no significant difference in the rates of absorption of glucose and glycerol from the gastrointestinal tract exists.

Another question is whether the rise in the concentration of blood sugar after glycerol is produced by an increase in the concentration of the blood. As previously noted, Voegtlin, Thompson, and Dunn[8] did not observe any increase in the concentration of the blood in rabbits after the ingestion of glycerol.

The point to be considered now is the capacity of the diabetic person to utilize glycerol as compared with glucose. As noted above, Sansum and Woodyatt[4] contend that glycerol does not have a greater utilization rate than glucose in diabetic persons.

The greatest glycemia, and consequently glycosuria, occurs in diabetic patients during the two- to three-hour period immediately following a meal. This degree of glycemia varies even when different hexoses are given. Thus galactose will give rise to a lower blood sugar curve than an equal amount of glucose. Consequently, the diabetic person can utilize galactose better than glucose. This is strikingly more so with glycerol. As already shown, the blood sugar curve when glycerol is ingested is markedly lower than when an equivalent amount of glucose is taken. It must be concluded then that the diabetic individual has a greater capacity to utilize glycerol than glucose.

It should be mentioned that this matter is of more scientific than practical importance, for the amount of glycerol derived by the saponification of fat is small. Furthermore, the glycerol is produced at a slow rate.

CONCLUSIONS

1. The ingestion of glycerol produces an increase in the concentration of blood sugar in a majority of normal and diabetic individuals.

2. Diabetic persons have a greater capacity to utilize glycerol than glucose.

REFERENCES

1. Catron, L. F., and Lewis, H. B.: Glycogen Formation From Glycerol, J. Biol. Chem. **84**: 553, 1929.
2. Shapiro, I.: The Comparative Glycogenic and Ketolytic Action of Glucose and Some Carbohydrate Intermediates, J. Biol. Chem. **108**: 373, 1935.
2a. Quoted by Shapiro, Ibid.
3. Chambers, W. H., and Deuel, H. J.: Metabolism of Glycerol in Phlorhizin Diabetes, J. Biol. Chem. **65**: 21, 1925.
4. Sansum, W. W., and Woodyatt, R. T.: The Behavior of dl-Glyceric Aldehyde in the Normal and Diabetic Organism, J. Biol. Chem. **24**: 327, 1916.
5. Voegtlin, C., and Dunn, E. R.: Antagonistic Action of Glycerol on Insulin Intoxication, J. Pharmacol. & Exper. Therap. **25**: 168, 1925.
6. Noble, E. C., and Macleod, J. J. R.: The Influence of Sugars and Other Substances on the Toxic Effects of Insulin, Am. J. Physiol. **64**: 547, 1923.
7. McCann, W. S., and Hannon, R. R.: Respiratory Exchange Following the Ingestion of Glucose, Glycerol, etc., Bull. Johns Hopkins Hosp. **34**: 73, 1923.
8. Voegtlin, C., Thompson, J. W., and Dunn, E. R.: Hyperglycemia Produced by Glycerol, J. Biol. Chem. **64**: 639, 1935.
9. Kneip, J.: Blutzuckerbeeinflussung durch perorale Fett-bzw. Glyzerinzufuhr bei kindern, Monatschr. f. Kinderh. **64**: 359, 1936.
10. Behrens, H. O.: Ueber den Einfluss des Glyzerin auf den Blutzuckerspiegel, Monatschr. f. Kinderh. **63**: 368, 1935.

GUANIDINE-LIKE SUBSTANCES IN BLOOD*

VII. Guanidine and Other Blood Constituents in Experimental Anoxemia

Jerome E. Andes, Ph.D., M.D., Edward J. Van Liere, Ph.D., M.D.,
Erlene J. Andes, B.S., M.S., and Paul Vaughn, B.S.,
Morgantown, W. Va.

IT HAS been pointed out in the previous paper of this series[1] that a tendency for hyperguanidinemia has been reported in a number of conditions exhibiting spasticity or convulsions. Although a part of these abnormal findings, or possibly all in some diseases, may be ascribed to faulty analytical methods or faulty interpretation, there is still sufficient data to make additional studies worth while in this direction.

As already pointed out[1] recent work seems to have entirely disproved the thesis of a hyperguanidinemia in parathyroid insufficiency.[2-8] The high values observed by previous investigators seem to be entirely due to faulty methods and

*From the Departments of Pathology and Physiology, West Virginia University School of Medicine, Morgantown.

Received for publication, November 20, 1939.

possibly to blood concentration from dehydration. There is more evidence, however, that the convulsive states associated with the toxemias of pregnancy and related disorders may have a definite relationship to the concentration of guanidine in the blood. Malmejac and co-workers,[9-11] Minot and Cutler,[12] and Andes, Andes, and Myers[13] have found a definite increase in the blood guanidine concentration in the toxemias of pregnancy, especially in true eclampsia. Traut and McFate[14] observed hyperguanidinemia in a case of dysmenorrhea accompanied by convulsions. The entirely negative findings of de Wesselow and Griffith,[15] Stander,[16] and Krieger[17] throw considerable doubt on the value of these findings. Andes and co-workers[13] have pointed out that hyperguanidinemia in eclampsia is usually accompanied by some degree of renal insufficiency, and suggest this factor as the probable cause of most of the increase (renal insufficiency is usually, if not invariably, accompanied by an increase in the blood guanidine concentration[18]). They did note, however, a definite hyperguanidinemia in two cases of eclampsia with convulsions not accompanied by nitrogen retention, and furthermore, pointed out that patients in convulsions tended to show higher values. They also demonstrated a definite increase in the blood guanidine in *normal* women after delivery.

Ellis, Neal, and Frazier[19] have reported an increased blood guanidine content in epileptic persons, the increase roughly paralleling the severity of the clinical symptoms. Although their work has not been confirmed, the changes observed are so marked as to apparently relate the blood guanidine change to the convulsant state. In addition, Dodd and Minot[20, 21] and also Schumacher,[22] have noted a hyperguanidinemia in the convulsive states of childhood. It is the opinion of some that dehydration is an important factor in producing the blood changes in this last set of conditions, but such has not been proved to be the entire cause.

In an attempt to elucidate the entire problem, Andes and Emerson[1] administered various convulsive drugs to rabbits and dogs, but obtained entirely negative results as far as blood guanidine was concerned. There still remained the possibility that anoxemia of the muscles and other tissues might be an important factor. Dordi's observation[23] of the presence of a hyperguanidinemia in pneumonia is some evidence as to the probability of this relationship being present (even though Dordi attributed the change to liver damage rather than to anoxemia). This work was carried out to determine whether any relationship existed between anoxemia per se and the guanidine content of the blood.

EXPERIMENTAL

Dogs were used as experimental animals. Only large, or moderately large, animals were selected to minimize as far as possible any error due to the slight degree of anemia resulting from the withdrawal of the relatively large blood samples (15 c.c.). The animals were fasted twenty-four hours before the experiment began and were not even allowed water during the course of the procedure.

Anoxemia was produced by the use of a tank in which the pressure and temperature could be held quite constant. The altitude in feet was computed by the barometric pressure inside the tank; likewise the partial pressure of oxygen. The period of anoxemia varied from thirty minutes to over seven and

TABLE I

BLOOD GUANIDINE IN ANOXEMIA

ANIMAL NO.	DATE	ALTITUDE IN FEET	PARTIAL PRESSURE OF O_2 MM. HG	TIME OF ANOXEMIA	UREA N MG. %	SUGAR MG. %	GUANIDINE MG. %	CO_2 CAPACITY VOL. %	HEMOGLOBIN %	CELL VOL. %
A. Using Separate Dog as Control										
1a	3/ 5/39	Control	150	None	12.9	154	0.68	39	15.8	
1b		18,000	80	45 min.	19.4	126	0.78	76	18.1	
2a	3/10/39	Control	150	None	12.6	156	0.31	37	11.9	
2b		18,000	80	30 min	8.1	136	0.34	39	13.0	
3a	3/14/39	Control	150	None	17.2	118	0.31	52	11.0	
3b		18,000	80	30 min	17.4	125	0.45	49	14.8	
4a	3/16/39	Control	150	None	16.4	122	0.44	34	16.4	
4b		24,000	63	30 min	21.6	136	0.63	42	18.7	
5a	3/18/39	Control	150	None	9.9	145	0.28	34	15.7	
5b		24,000	63	30 min	10.8	152	0.56	36	18.5	
6a	3/23/39	Control	150	None	15.7	152	0.61	44	16.5	
6b		24,000	63	30 min	13.3	143	0.36	38	14.7	
7a	4/ 8/39	Control	150	None	9.5	204	0.59	38	15.1	
7b		28,000	53	30 min	11.0	172	0.68	24	14.8	
8a	4/14/39	Control	150	None	15.0	105	0.56	33	16.4	
8b		28,000	53	30 min	14.0	159	0.57	44	16.3	
Average		Control			13.5	151	0.47	39	14.8	
		anoxemic			16.2	144	0.55	44	16.1	
B. Using Same Animal as Control										
9	7/18/39	Control	150	None	13.3	74	0.70	36	12.5	36
		18,000	63	4 hr.	15.0	69	0.62	40	14.6	39
10	7/19/39	Control	150	None	9.8	105	0.67	40	14.5	39
		18,000	63	4 hr.	10.5	80	0.65	34	15.2	41
11	7/19/39	Control	150	None	23.1	74	0.59	41	17.1	45
		18,000	63	4 hr.	33.3	75	0.54	32	17.3	45
12	7/21/39	Control	150	None	9.8	67	0.74	29	14.9	40
		24,000	63	4 hr.	13.1	69	0.67	27	14.5	39
13	7/21/39	Control	150	None	11.0	77	0.68	42	15.8	41
		24,000	63	4 hr.	11.6	87	0.66	36	15.7	42
14	7/22/39	Control	150	None	13.3	74	0.70	36	12.8	36
		24,000	63	4½ hr.	21.0	81	0.68	28	14.7	38
15	7/24/39	Control	150	None	9.8	105	0.67	40	14.5	39
		24,000	63	7½ hr.	12.1	86	0.81	21	15.5	44
16	7/24/39	Control	150	None	23.1	74	0.59	41	17.1	45
		24,000	63	7½ hr.	18.4	83	0.64	35	17.5	47
Average (Nos. 9-16)		Control			14.2	79	0.66	38	14.9	40
		anoxemic			17.0	82	0.66	32	15.6	42
Average of all		Control			13.3	113	0.57	39	14.9	40
		anoxemic			16.0	111	0.60	38	15.9	42
		Change			+3.7	--	+0.03	--	1.0	+2

one-half hours. The altitudes used varied from 18,000 to 24,000 feet (oxygen pressures of 80 to 53 mm. Hg, respectively). It is believed that this degree of anoxemia (especially in the seven-and-one-half-hour periods) is entirely ample to bring out any alterations in the blood chemistry that might be observed in in life during anoxemia resulting from a spastic state.

In the first group of animals two dogs were used for each experiment: one as a control and one for the anoxemic experiment. Both dogs were anesthetized with sodium barbital and sacrificed after the experiment. The use of a separate control was decided upon to eliminate the possible error from blood loss due to the withdrawal of blood samples. The anesthetic served to obviate errors arising from muscular activity and dehydration during the period of anoxemia. In the second group of animals the same dog was used as a control, that is, samples were withdrawn before and after the period of anoxemia. No anesthetic was employed in this group and only relatively large animals were chosen.

Blood sugar, urea, and carbon dioxide capacity were determined by standard analytical procedures. Blood guanidine was estimated by the method of Andes and Myers.[24] Hemoglobin was measured by both photoelectric and colorimetric procedures. The determinations were all made immediately after the blood was withdrawn. The results are given in Table I. The table is divided into two parts in order to separate the two groups of animals.

DISCUSSION OF RESULTS

The findings are essentially negative in both groups of animals except for the blood urea, hemoglobin, and cell volume. The blood urea increase is significant although small. No explanation can be given for this change other than the effect of anoxemia on kidney function. The amount of hemoconcentration present, as denoted by the values for hemoglobin and cell volume, was too small to account for all this increase in the concentration of blood urea. The changes in the hemoglobin and cell volume were about what one would expect from the degree of anoxemia present. The values for guanidine and blood sugar were not significantly changed by the experiment. It will be noted that the glucose content of the blood is abnormally high in the group of animals in part A, Table I. This is presumably due to the fact that these animals received ether prior to the administration of barbital. The lack of further rise of blood sugar in the experimental animals may be due to the inhibiting effect of barbiturates on epinephrine release which would otherwise be produced by anoxia. The group of animals in part B, Table I received no anesthesia. Here again there is a lack of hyperglycemic response to anoxia. It must be pointed out, however, that the blood samples were drawn after four hours or more of anoxia.

The fall in the carbon dioxide capacity in the second set of animals is significant, but it is no more than one would expect from the amount of hyperpnea produced by the lack of oxygen and the additional hours of starvation. In the first group of animals the values for the carbon dioxide capacity are too erratic to be of any significance.

SUMMARY

1. Under the conditions described in this paper definite increases in the values of blood urea, hemoglobin, and volume of red blood cells were observed in anoxemic dogs. The guanidine content in the blood, however, was unchanged.

2. Some factor other than anoxemia must be sought as the cause of the hyperguanidinemia found in convulsive states.

REFERENCES

1. Andes, J. E., and Emerson, G. A.: Guanidine-Like Substances in Blood. VI. Blood Guanidine After Administering Convulsant Drugs, Arch. internat. de pharmacodyn et de thérap. **40**: 30, 1938.
2. Andes, J. E., and Myers, V. C.: Guanidine-Like Substances in Blood. IV. Blood Guanidine in Patients With Parathyroid Deficiency and With Idiopathic Tetany, J. LAB. & CLIN. MED. **23**: 123, 1937.
3. Berencsy, G., and Zinhobel, K.: Über einige gegen die Guanidintheorie spreckende Versuche, Klin. Wchnschr. **9**: 1503, 1930.
4. Bryan, W. R., Minot, A. S., and Chantain, L. L.: Hyperguanidinemia Associated With Dehydration in Normal and Parathyroidectomized Dogs, Am. J. Physiol. **106**: 738, 1933.
5. Major, R. H., Orr, T. G., and Weber, C. J.: Observations on the Blood Guanidine in Tetania Parathyreopriva, Bull. Johns Hopkins Hosp. **40**: 287, 1927.
6. Saunders, J. A.: Guanidine and the Parathyroid Glands, Biochem. J. **29**: 1597, 1935.
7. Swingle, W. W., and Nicholas, J. S.: The Effect of Chemical Compounds on the Production of the Tetany Syndrome, Am. J. Physiol. **69**: 455, 1924.
8. von Beznak, A., and Szeöke, K.: Über den Zusammenhang von Guanidinstoffwechsel and Aktivität der Leberarginase bei parathyreopriv tetanischen Hunden, Biochem. Ztschr. **239**: 159, 1931.
9. Laffont, A., Malmejac, R., and Sirjean: Guanidinemia and Gestation, Ann. de méd. phys. **6**: 395, 1930.
10. Malmejac, J.: Guanidinémia et intoxication gravidique, Rev. franç. de gynéc. et d'obst. **25**: 435, 1930.
11. Malmejac, R.: Las rapports de la guanidinémia avec la puerpéralité, Gynéc. et obst. **24**: 689, 1931.
12. Minot, A. S., and Cutler, J. T.: Increase in Guanidine-Like Substance in Acute Liver Injury, Proc. Soc. Exper. Biol. & Med. **26**: 607, 1929.
13. Andes, J. E., Andes, E. J., and Myers, V. C.: Guanidine-Like Substances in the Blood. III. Blood Guanidine in Normal Pregnancy, Toxemias of Pregnancy, and Cirrhosis of the Liver, J. LAB. & CLIN. MED. **23**: 9, 1937.
14. Traut, E. F., and McFate, R. P.: Hyperguanidinemia in Tetany, J. A. M. A. **96**: 266, 1931.
15. de Wesselow, O. L. V. S., and Griffiths, W. J.: Blood Guanidine in Hypertension, Brit. J. Exper. Path. **13**: 428, 1932.
16. Stander, H. J.: Guanidine Base Concentration in Eclampsia, Am. J. Obst. & Gynec. **23**: 373, 1932.
17. Krieger, V. I.: Observations on Guanidine, Glucose and Calcium Content of Blood in Eclampsia, M. J. Australia **2**: 746, 1934.
18. Andes, J. E., Linegar, C. R., and Myers, V. C.: Guanidine-Like Substances in the Blood. II. Blood Guanidine in Nitrogen Retention and Hypertension, J. LAB. & CLIN. MED. **22**: 1209, 1937.
19. Ellis, M. M., Neal, M. P., and Frazier, T. R.: Guanidine Content of Blood From Epileptics, Proc. Soc. Exper. Biol. & Med. **28**: 553, 1931.
20. Dodd, K., Minot, A. S., and Casparis, J.: Guanidine as a Factor in Alimentary Intoxication in Infants, Am. J. Dis. Child. **43**: 1, 1932.
21. Dodd, K., and Minot, A. S.: Hyperguanidinemia and Tetany, Am. J. Dis. Child. **47**: 958, 1934.
22. Schumacher, H. M.: Über die Beziehung des Guanidins zur Tetanie, Ztschr. f. Kinderh. **56**: 307, 1934.
23. Dordi, A. M.: Guanidine in Blood in Diseases of the Respiratory Tract in Children, Riv. di clin. pediat. **37**: 448, 1939.
24. Andes, J. E., and Myers, V. C.: Guanidine-Like Substances in Blood. I. Colorimetric Estimation and Normal Values, J. LAB. & CLIN. MED. **22**: 1147, 1937.

A COMPARISON OF THE CHLORIDE CONCENTRATIONS IN WHOLE BLOOD AND SPINAL FLUID*

ROGER S. HUBBARD, PH.D., AND GILBERT M. BECK, M.D.
BUFFALO, N. Y.

IT IS generally believed that spinal fluid is formed from the fluids and solutes of the blood, and that the composition of the former medium reflects more or less closely that of the latter.[1] Comparative studies of these fluids have been extensively carried out in this country by Fremont-Smith and various co-workers.[2-6] In part as a result of these and similar studies, the thesis has been developed that spinal fluid can be properly regarded as a modified dialysate of plasma. As this thesis has been adequately presented and discussed in various recent textbooks,[7-9] a review of the history of the problem will not be given here.

The majority of the papers which have dealt with this subject have had for their purpose an examination of the evidence favoring or criticising the thesis just stated. This has led previous investigators to carry out their determinations under certain specified conditions. Since the spinal fluid is apparently, at least in part, a dialysate of blood plasma,[1, 10] precautions have been taken to secure specimens for analysis which shall resemble as closely as possible plasma as it exists in the body—the so-called "true plasma." Blood has been collected without stasis under oil, and the cells removed from the specimen by centrifuging without contact with air. These precautions are taken to avoid changes in the carbon dioxide tension and the migration of chloride ions between the cells and plasma, that is, the so-called "chloride shift," which results from changes in this tension.[11] Specimens of blood and fluid have regularly been drawn after a night's fast when the effects of the ingestion of food and water are at a minimum.

Since the determination of the chloride content of the spinal fluid has been found to be of value in the diagnosis of meningitis, particularly of tubercular meningitis,[1, 14, 15] such analyses are frequently requested of clinical chemical laboratories. It seemed desirable to analyze blood and spinal fluid taken simultaneously in order to call the clinician's attention to the presence of a lowered chloride in the blood when such existed. This should decrease any tendency to attach too much specificity to the results of the spinal fluid analyses. It was hoped also that such a procedure would make it possible to detect the effect upon the spinal fluid chloride concentration of factors not associated with variations in the blood, such as changes in the protein concentration of the spinal fluid[2, 4] and possibly of the replacement of chloride by lactic acid formed during the destruction of sugar by organisms and enzymes.[2]

Soon after this program was instituted it was found that these specimens of spinal fluid submitted for analysis frequently were taken from patients not in the postabsorptive state. It was also recognized that under the conditions existing in the clinic, the samples of blood obtained were as a rule not suitable

*From the Buffalo General Hospital and the Medical School of the University of Buffalo. Received for publication, March 11, 1940.

for the preparation of "true plasma." The procedure was, therefore, definitely modified from that followed by Fremont-Smith and his co-workers. Specimens were drawn at any time during the day, and the whole blood was chosen as the material to be used for the control analyses. The method of Whitehorn[16] was used for the determination of chlorides in both blood and fluid.

It was recognized that this procedure was not as well adapted to the study of the physiologic problem involved as was that employed by the investigators already cited. However, it was felt that one principal source of error, that due to variations in the concentration of red blood cells which are relatively low in chloride, could be controlled by routine blood counts. The other chief sources of error, variations in the chloride concentration in the red blood cells, and failure of the spinal fluid and blood to reach a condition of approximate equilibrium, were necessarily ignored.

We were not able to find any data which might serve as a base line to determine the usual ratio between the whole blood and spinal fluid chloride concentrations. We have, therefore, undertaken to establish such a standard by statistical analysis of our own material. Because we feel that other clinics are probably confronted by problems similar to our own, we are presenting the results for publication. In order to compare the relationship with that obtained under more nearly ideal conditions, we have analyzed the 331 determinations made upon true plasma and spinal fluid, obtained in the postabsorptive condition, published by Fremont-Smith and his co-workers.[5, 6] The character of the clinical material upon which these earlier studies were based seems to have been similar to that from the wards and out-patient department of the Buffalo General Hospital, but the number of normal cases was apparently somewhat greater in the series of the plasma determinations. To facilitate comparison of the two sets of data the results have been expressed according to standard statistical methods.

Table I shows the distribution of the separate values for whole blood and spinal fluid of the 225 analyses made at the Buffalo General Hospital. Inspection of the table shows that there was a marked correlation between the chloride content of the two fluids in the various specimens. The values of the ratio $\frac{\text{spinal fluid chloride}}{\text{whole blood chloride}}$ are given in the table. These were neither absolutely constant nor wholly independent of the blood and spinal fluid chlorides. They apparently tended to increase slightly as the concentration of chloride in the spinal fluid increased, and to decrease as the concentration in the blood increased. The data on the chloride content of the plasma and the spinal fluid presented in the papers of Fremont-Smith and his co-workers are given in Table IA. It is evident that the range of values is much less than that shown by our own results. The difference between the ranges of the whole blood and of the plasma figures is readily explained, for the composition of the plasma is much more nearly constant than is that of blood, as variations in the number of red blood cells, containing relatively small amounts of chloride, do not affect the latter figure. Two factors appear to have produced the difference between the distributions of the spinal fluid values in the two series. One of these probably was a difference in the method of selection of the cases studied; the other was the

TABLE I

WHOLE BLOOD CHLORIDE AND SPINAL FLUID CHLORIDE VALUES—225 DETERMINATIONS

(Buffalo General Hospital)

The values of the ratio $\frac{\text{spinal fluid chloride}}{\text{whole blood chloride}}$ have been multiplied by 100 and expressed as per cent.

SPINAL FLUID MG. NaCl/100 C.C. RANGE	WHOLE BLOOD—MG. NaCl PER 100 C.C.—RANGE OF VALUES								RATIO PER CENT
	251-300	301-350	351-400	401-450	451-500	501-550	551-600	TOTAL	
401-450	0	2	0	0	0	0	0	2	142.5
451-500	2	1	1	0	0	0	0	4	157.5
501-550	0	4	3	1	0	0	0	8	156.3
551-600	0	2	14	9	0	0	0	25	151.2
601-650	1	0	10	23	3	1	0	38	149.1
651-700	0	0	2	36	36	2	0	76	152.0
701-750	0	0	1	19	36	5	0	61	155.7
751-800	0	0	0	5	4	0	1	10	163.5
801-850	0	0	0	0	0	0	0	0	--
851-900	0	0	0	0	0	0	0	0	--
901-950	0	0	0	0	0	0	1	1	156.0
Total	3	9	31	93	79	8	2	225	
Ratio per cent	190.0	157.0	152.7	156.3	148.9	134.3	145.5		153.6

TABLE IA

PLASMA CHLORIDE AND SPINAL FLUID CHLORIDE VALUES—331 DETERMINATIONS

(Fremont-Smith and Co-workers)

The values of the ratio $\frac{\text{spinal fluid chloride}}{\text{plasma chloride}}$ have been multiplied by 100 and expressed as per cent.

SPINAL FLUID MG. NaCl/100 C.C. RANGE	PLASMA—MG. NaCl PER 100 C.C.—RANGE OF VALUES								RATIO PER CENT
	316-364	365-413	414-462	463-511	512-560	561-609	610-658	TOTAL	
498-546	0	0	2	0	1	0	0	3	114.8
547-595	1	1	0	7	3	0	0	12	122.7
596-644	0	0	1	8	17	1	0	27	119.8
645-693	0	0	1	6	34	33	1	75	123.4
694-742	0	0	0	2	11	163	21	197	122.7
743-791	0	0	0	0	0	7	10	17	123.3
Total	1	1	4	23	66	204	32	331	
Ratio per cent	171.5	151.5	133.5	126.5	122.4	122.2	122.2		122.6

fact that Fremont-Smith's studies were made upon specimens obtained in the postabsorptive state when there is relatively little variation in the composition of all body fluids. In our own series the effect of the ingestion of water and of salt was unquestionably marked in some instances. Except for the difference in the "spread" of the figures, Table IA bears a close resemblance to Table I. The correlation between the plasma and spinal fluid chloride values is marked, and the values of the ratio $\frac{\text{spinal fluid chloride}}{\text{plasma chloride}}$ tend to vary directly as the spinal fluid values, and inversely as the plasma chloride concentrations.

The correlation coefficients based upon these analyses are given in Table II. In both series the correlation between the chloride of the spinal fluid and the chloride of the plasma and of the whole blood was positive and marked. The parallelism between the spinal fluid and plasma chlorides was closer than was

TABLE II

CORRELATION COEFFICIENTS

	BLOOD OR PLASMA NaCl AND SPINAL FLUID NaCl	BLOOD OR PLASMA NaCl AND RATIO	SPINAL FLUID NaCl AND RATIO
Fremont-Smith Plasma 331 determinations	+0.811 ± 0.013	−0.467 ± 0.029	+0.091 ± 0.037
Hubbard and Beck Whole blood 225 determinations	+0.766 ± 0.018	−0.483 ± 0.051	+0.118 ± 0.044

that between the spinal fluid and the whole blood chlorides, as would be expected from the probable mechanism involved for the spinal fluid is apparently formed directly from the plasma. The existence of a slight positive correlation between the values of the ratio $\frac{\text{spinal fluid chloride}}{\text{whole blood (or plasma) chloride}}$ and of spinal fluid chloride suggested in Tables I and IA is confirmed to some extent by the correlation coefficients. Analysis of both series of data gave slight positive coefficients, which were probably, but not certainly, significant. We believe that this parallelism results from the relationship between the amount of protein and of chloride in the spinal fluid discussed previously by Merritt and Fremont-Smith,[9] and shown for our own data in Table III. If the protein concentration affects directly the spinal fluid chloride, and is itself independent of variations in the chloride of the blood and plasma, values of the ratio should parallel values of the spinal fluid chloride, i.e., variations in the spinal fluid chloride will to some extent, be independent of variations in the blood chloride concentration.

Table II also shows that a significant negative correlation existed between the values of the ratio and the chloride concentration of the whole blood or plasma. While a slight negative correlation might arise from the method by which the ratio is calculated (for increases in the denominator of a fraction must have some tendency to cause decreases of the value of the fraction), it seems to us that the correlation is too close to be explained wholly in this way. We believe that variations in the plasma and the whole blood chloride sometimes do not cause changes of the usual magnitude in the concentration of that substance in the spinal fluid. One factor, and obviously an important one, has been discussed by Fremont-Smith.[5] He suggested that it takes a fairly long period of time to establish an equilibrium between the chlorides in the blood and the spinal fluid. This was in accord with previous work, for it had already been shown that an equilibrium between the glucose concentration in the two fluids is reached rather slowly.[18] He pointed out that the most pronounced divergence in values of the ratio from those expected occurred in patients who were losing chloride and water by emesis or who were receiving either chloride or water therapy. In our own series the same type of patient showed similar unusual relationships. It was noted, too, that in many instances ratios which deviated markedly from the average of the series were obtained on individuals who were showing marked variations in the chloride content of the spinal fluid. Such an "abnormal" ratio was not infrequently preceded or followed by an entirely "normal" ratio within one or two days. This strongly suggests that some delay in attaining equilibrium

TABLE III

CORRELATION COEFFICIENTS

COMPARISON OF SPINAL FLUID PROTEIN WITH BLOOD AND SPINAL FLUID CHLORIDES

(Hubbard and Beck—58 Determinations)

BLOOD NaCl AND SPINAL FLUID NaCl	BLOOD NaCl AND RATIO	SPINAL FLUID NaCl AND RATIO	SPINAL FLUID PROTEIN AND BLOOD NaCl	SPINAL FLUID PROTEIN AND SPINAL FLUID NaCl	SPINAL FLUID PROTEIN AND RATIO
+0.812 ± 0.030	−0.431 ± 0.072	+0.187 ± 0.084	+0.040 ± 0.080	−0.309 ± 0.080	−0.416 ± 0.073

TABLE IV

STATISTICAL CONSTANTS

Results of analyses made upon whole blood, plasma, and spinal fluid are expressed as milligrams of sodium chloride per 100 c.c.

SOURCE OF DATA AND FACTORS	MEAN MG./100 C.C.	MEDIAN MG./100 C.C.	STANDARD DEVIATION MG./100 C.C.	COEFFICIENT OF VARIATION PER CENT
Fremont-Smith—331 specimens				
Plasma NaCl	570.3 ± 1.5	578.6 ± 1.9	40.80 ± 1.07	7.15 ± 0.19
Spinal fluid NaCl	698.0 ± 1.6	713.6 ± 2.0	43.17 ± 1.13	6.18 ± 0.16
Ratio $\frac{\text{spinal fluid NaCl}}{\text{plasma NaCl}}$	1.226 ± 0.002	1.224 ± 0.003	0.0622 ± 0.0016	5.08 ± 0.13
Hubbard and Beck—225 specimens				
Whole blood NaCl	433.9 ± 2.2	442.1 ± 2.7	48.58 ± 1.54	11.19 ± 0.36
Spinal fluid NaCl	664.1 ± 3.0	682.5 ± 3.7	66.41 ± 2.11	10.00 ± 0.32
Ratio $\frac{\text{spinal fluid NaCl}}{\text{whole blood NaCl}}$	1.536 ± 0.005	1.525 ± 0.008	0.1187 ± 0.0034	7.73 ± 0.25
Spinal fluid protein—58 specimens				
Whole blood NaCl	423.6 ± 5.0	430.5 ± 6.25	56.46 ± 3.53	13.33 ± 0.84
Spinal fluid NaCl	642.7 ± 7.4	649.1 ± 9.3	76.41 ± 4.80	11.89 ± 0.74
Spinal fluid protein	0.224 ± 0.021	0.105 ± 0.026	0.237 ± 0.015	104.29 ± 6.53
Ratio $\frac{\text{spinal fluid NaCl}}{\text{blood NaCl}}$	1.522 ± 0.012	1.498 ± 0.015	0.1372 ± 0.0086	9.01 ± 0.58

had affected the results. It is, of course, not possible to decide whether the occurrence of an unusual relationship between the chlorides in blood and spinal fluid always result from such a simple cause as this, or whether more specific factors must sometimes be considered in explaining the lack of constancy of the ratio $\frac{\text{spinal fluid chloride}}{\text{blood (or plasma) chloride}}$.[10]

It has been stated that there is evidence that the protein content of the spinal fluid has a direct effect upon the concentration of the chloride. Protein determinations were made by the method of Hubbard and Garbutt[18] upon 58 of the 225 specimens of spinal fluid upon which this study is based. A statistical analysis of the results is shown in Table III. The correlation coefficients of the small series do not differ greatly from those of the larger one of which it forms a part. There was no relationship between the protein in the spinal fluid and the concentration of chloride in the blood. There were negative correlations between the spinal fluid protein on the one hand and the spinal fluid chloride and the values of the ratio $\frac{\text{spinal fluid chloride}}{\text{whole blood chloride}}$

on the other hand. Such a relationship would be expected if the spinal fluid protein causes, or is fairly regularly accompanied by, decreases in the spinal fluid chloride. Such decreases may result from a diminution of the usual difference between the protein concentrations of plasma and spinal fluid.[7]

The usual statistical constants for the figures obtained in our own series, for those calculated from the data of Fremont-Smith, and for the 58 cases upon which protein analyses were available are given in Table IV. There is need of only brief comment upon this table. The blood and spinal fluid chloride values showed a high degree of variability and gave distribution curves which departed markedly from the symmetrical form, as shown by the large difference between the mean and median values. The ratios calculated from these observed data showed relatively little variability, and, while not perfectly symmetrical, were nearly so. As expected, the values of ratios calculated from the data of Fremont-Smith, based upon analyses of true plasma, appeared to be more nearly constant and to show more symmetrical distribution than did those of ours based upon

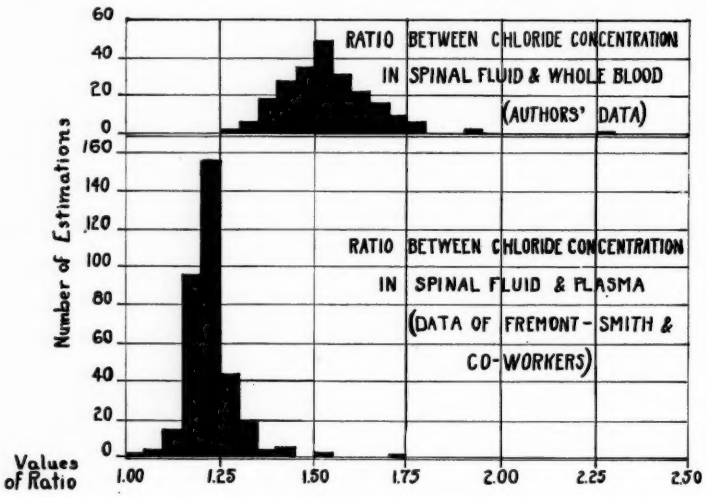

Chart 1.

determinations of the whole blood chloride. The difference between the two sets of values was not as great, however, as we had expected it would be.

Since it is hoped that the results of this study will be of value to other clinics in which spinal fluid analyses are carried out, Chart 1 has been prepared to show the distribution of the values of the ratios $\frac{\text{spinal fluid chloride}}{\text{whole blood chloride}}$ and $\frac{\text{spinal fluid chloride}}{\text{plasma chloride}}$. The chart shows graphically the distribution indicated in statistical terms in Table IV. These ratios show such constancy that either may be useful in checking the probable significance of spinal fluid analyses. The results based upon studies of true plasma were obviously somewhat more satisfactory than were those based upon analyses of whole blood.

CONCLUSIONS

Statistical analysis of a series of simultaneous determinations of whole blood chloride and spinal fluid chloride shows that a high degree of correlation exists

between the two sets of values. While the agreement of the spinal fluid chloride with the whole blood chloride was not as close as was the agreement of the former with plasma concentration, the agreement was nevertheless close enough to show that simultaneous determinations of the chloride content of whole blood can serve as a useful check upon the significance of spinal fluid analyses. Such controls should be especially valuable in clinics where the more complicated procedures cannot be readily carried out. It could be shown that three factors influence the value of the ratio between spinal fluid and whole blood chloride: (1) the protein content of the spinal fluid; (2) the concentration of cells in the blood; (3) the failure of the blood and spinal fluid to reach equilibrium when the fluid and salt content of the body were rapidly changing. Whether any other factor or factors influence the equilibrium, and so specifically affect the concentration of chloride in the spinal fluid, could not be determined.

REFERENCES

1. Mesetrat, W.: Le liquide cephalo-rachidien, Paris, 1912, A. Maloine et fils.
2. Fremont-Smith, F., and Dailey, M. E.: Cerebrospinal Fluid Chlorides, Arch. Neurol. & Psych. **14**: 509, 1925.
3. Fremont-Smith, F., and Dailey, M. E.: The Effect of Protein Concentration Upon the Chloride Equilibrium Between Plasma and Cerebrospinal Fluid, J. Pharmacol. & Exper. Therap. **27**: 255, 1926.
4. Fremont-Smith, F., and Dailey, M. E.: Studies in the Distribution of Chloride and Protein Between Plasma and Synovial Fluid, J. Biol. Chem. **70**: 779, 1926.
5. Fremont-Smith, F., and Dailey, M. E., Merritt, H. H., Carroll, M. P., and Thomas, G. W.: The Equilibrium Between Blood and Spinal Fluid. I. The Composition of the Human Cerebrospinal Fluid and Plasma, Arch. Neurol. & Psych. **25**: 1271, 1931.
6. Fremont-Smith, F., and Dailey, M. E., Merritt, H. H., and Carroll, M. P.: The Equilibrium Between Cerebrospinal Fluid and Blood Plasma. II. The Composition of the Human Cerebrospinal Fluid and Blood Plasma in Meningitis, Arch. Neurol. & Psych. **25**: 1290, 1931.
7. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Vol. I. Interpretations, Baltimore, 1931, p. 1075.
8. Katzenelenbogen, S.: The Cerebrospinal Fluid and Its Relation to the Blood, Baltimore, 1935, p. 101.
9. Merritt, H. H., and Fremont-Smith, F.: The Cerebrospinal Fluid, Philadelphia, 1937, p. 20.
10. Fremont-Smith, F.: The Nature of Cerebrospinal Fluid, Arch. Neurol. & Psych. **17**: 317, 1927.
11. Best, C. H., and Taylor, M. B.: The Physiological Basis of Medical Practice, Baltimore, 1937, p. 168.
12. Fremont-Smith, F., and Dailey, M. E.: Cerebrospinal Fluid Sugar, Arch. Neurol. & Psych. **14**: 390, 1925.
13. Hubbard, R. S., and Allison, C. B.: An Apparent Effect of Normal Variations in the Respiratory Rate Upon the Excretion of Chloride and Water, J. Biol. Chem. **89**: 627, 1930.
14. Nobecourt, P., and Voisin, R.: Les chlorures de liquide cephalo-rachidien dans divers etats pathologiques et en particulier dans le bronchopneumonies infantile, Arch. gén. de méd. **2**: 3018, 1903.
15. Neuda, P.: Die praktische und theoretische Bedeutung der Chlorbestimmung im liquor cerebrospinalis, Wien. klin. Wchnschr. **36**: 447, 1923.
16. Whitehorn, J. C.: System of Blood Analysis, J. Biol. Chem. **45**: 449, 1921.
17. Fremont-Smith, F., and Dailey, M. E.: Cerebrospinal Fluid Sugar, Arch. Neurol. & Psych. **14**: 390, 1925.
18. Hubbard, R. S., and Garbutt, H. R.: The Determination of Protein in Cerebrospinal Fluid, Am. J. Clin. Pathol. **5**: 433, 1935.
19. Best, C. H., and Taylor, N. B.: The Physiological Basis of Medical Practice, Baltimore, 1937, p. 1492.

LABORATORY METHODS

GENERAL

A STANDARDIZED TECHNIQUE FOR SEDIMENTATION RATE*

J. W. CUTLER, M.D., PHILADELPHIA, PA.

LACK of a universally accepted technique for recording erythrocyte sedimentation rate has been a serious block to a more widespread adoption of this simple test in general practice. The great majority of techniques described have complicated the problem, making comparison of results by the various procedures difficult.

All techniques described were originally developed empirically. In each the unit of comparison (usually the total drop at the end of one hour expressed in millimeters), while it reflects the rapidity of sedimentation in a general way, fails to give an accurate idea of the rate of settling. Furthermore, an arbitrary determination at the end of a stated interval, such as one hour, also reflects anemia because of the packing of the cells in the bottom of the tube within the first hour in rapidly settling blood. Efforts to "correct" for anemia, as advocated by some investigators, have failed to yield consistent sedimentation findings.

The object of the present paper is to describe a technique that is based on principles applicable to all tubes. The method is simple, and requires no correction for anemia. In a way this technique is a modification of the previous Cutler method, but the approach and interpretation are totally different. To understand it, it is necessary to keep in mind the present concept of the factors believed responsible for the rapid settling of the erythrocytes.

It is generally accepted that rapid sedimentation is dependent upon the formation of large aggregates or rouleaux of red blood cells and that rouleaux once formed settle at a given rate of speed, more or less in accordance with Stokes' law of hydrodynamics—the larger the aggregate the more rapid the settling and vice versa (Figs. 1 and 2). It has been established that the arrangement of the red blood cells in rouleaux and the size of the rouleaux are effects brought about almost entirely by the plasma and are specific for that plasma, and that the size and number of cells in suspension, as in anemia, have little influence on this specificity of the plasma.[1] The exact nature of the changes in the plasma which bring about the formation of large rouleaux is not known, but it is generally believed that the hydrophilic substances present in the plasma, i.e., the proteins which vary in type and electrolyte combination, play

*From the Henry Phipps Institute and Graduate Hospital of the University of Pennsylvania, Philadelphia.
Presented before the Pathological Society of Philadelphia, January 11, 1940.
Received for publication, April 2, 1940.

a predominant role, probably through surface dehydration or change in the water balance on the surface of the red blood cells.

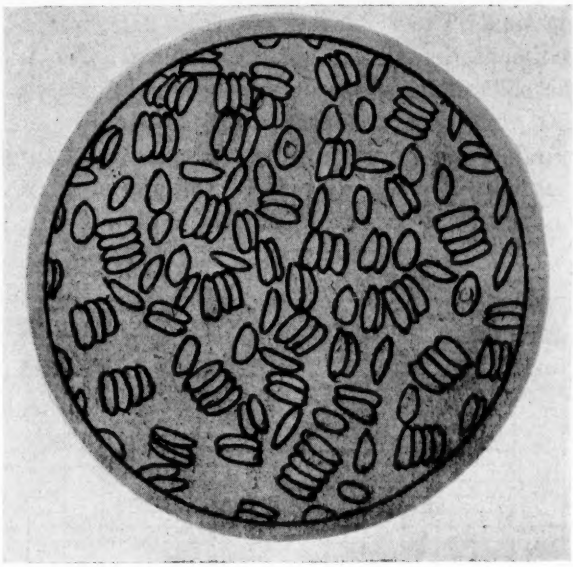

Fig. 1.—Rouleaux formation in slowly settling blood. The rouleaux are small and consist of relatively few cells.

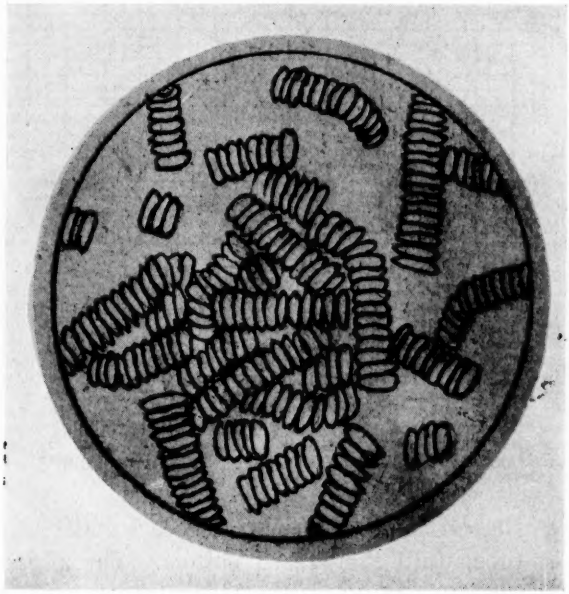

Fig. 2.—Rouleaux formation in rapidly settling blood. The rouleaux are large and consist of many cells. It is the increased mass of the large rouleaux that brings about rapid sedimentation. This fundamental fact underlies the technique for determining sedimentation rate described in the text.

THREE PHASES OF ERYTHROCYTE SEDIMENTATION

We are now in a position to study in detail the sedimentation phenomenon as recorded in the form of graphs. Four distinct types of graphs, a horizontal

line, a diagonal line, a diagonal curve, and a vertical curve (Fig. 3), have been recognized.[2] The horizontal line is normal and denotes slow sedimentation. The other graphs are abnormal findings and indicate various degrees of increased sedimentation. The vertical curve (Fig. 3*D*), which indicates the most rapid form of sedimentation, consists of three phases. The first is a slow phase, during which the cells are grouping themselves and forming aggregates. This aggregation period usually varies from five to fifteen minutes; occasionally it is as long as thirty minutes. Once formed, the aggregates fall uniformly, more or less in accordance with Stokes' law—the larger the aggregate the more rapid the fall. This is the second or settling phase and gives rise to the sedimentation phenomenon.

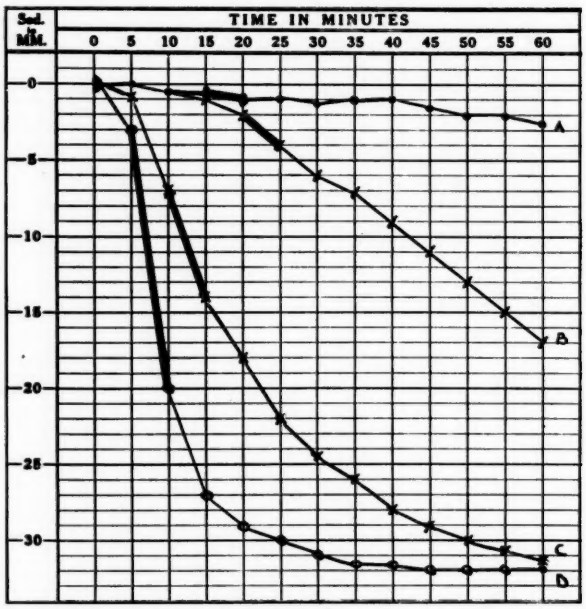

Fig. 3.—Typical graphs depicting erythrocyte sedimentation. *A*, horizontal line; *B*, diagonal line; *C*, diagonal curve; *D*, vertical curve. Only the horizontal line is normal.

When the retarding influence of packing is absent, the rate of settling of the rouleaux, once formed, is fairly uniform; this is best illustrated in graph *B*, where the rate is 2 mm. every five minutes.

The heavy part of each graph denotes the maximum settling in any five minutes during the hour and is a better guide to sedimentation rate than the sedimentation index (total drop at the end of one hour). Thus, graphs *C* and *D* have practically the same index (32 and 33 mm., respectively) but strikingly different maximum rates in five minutes (7 and 17 mm., respectively). Graph *C* is from a patient in her ninth month of pregnancy; graph *D* is from a patient with fatal carcinoma of the lung. Note that the maximum sedimentation rate in five minutes almost invariably occurs during the first thirty minutes and that no readings need be taken with the new technique beyond that time.

The aggregates settle at their respective speeds indefinitely, depending on the length of the tube, until they reach bottom. The aggregates that follow pack on top, and the packing stage, or third phase, of sedimentation sets in. Sedimentation during the packing stage is always slow. When all aggregates have settled tightly, sedimentation comes to a standstill.

It is apparent that by the time packing ensues, the sedimentation phenomenon has already taken place and that the packing of the cells represents only a rough hematocrit finding, recording the space in the tube occupied by the cells. The packing stage is the only phase of sedimentation that is ma-

terially influenced by the degree of anemia. The fewer the cells, the less volume they occupy.

The three phases, aggregation, sedimentation, and packing, are found in all graphs, in the horizontal normal as well as in the definitely abnormal vertical curve. The difference is in the time required to complete the second stage, or stage of sedimentation which depends upon the mass of the aggregates formed during the first or aggregation phase, which in turn is a function of the plasma. To study the sedimentation phenomenon, one should be concerned principally with the second phase, or phase of settling.

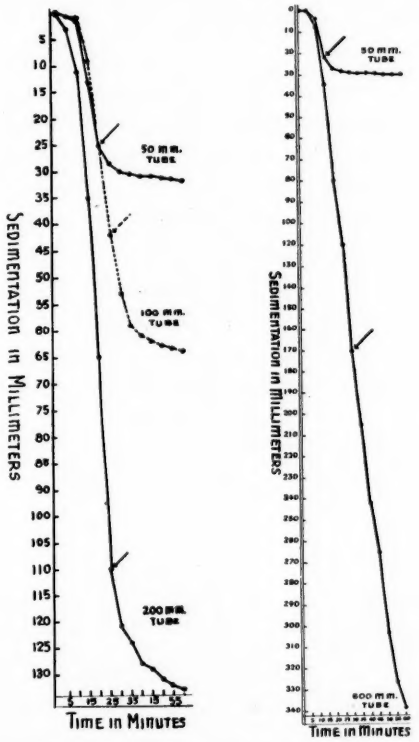

Fig. 4.—The influence of packing of the cells in the bottom of the tube (packing) on the rapidity of sedimentation in tubes 50, 100, 200, and 600 mm. long, respectively, and 5 mm. internal diameter. Readings every five minutes during the first hour (same sample of blood). In the first ten or fifteen minutes settling is slow in all tubes, for it is during this period that the red blood cells group themselves into rouleaux. Once rouleaux are formed, however, settling becomes rapid. Sedimentation reaches maximum 12 mm. in five minutes in the 50 mm. tube between fifteen and twenty minutes; 17 mm. in five minutes in the 100 mm. tube between twenty and twenty-five minutes; 45 mm. in five minutes between twenty and twenty-five minutes in the 200 mm. tube; and 50 mm. in five minutes between twenty-five and thirty minutes in the 600 mm. tube. From that point on (indicated by arrows) sedimentation in five-minute periods becomes progressively slower in each tube because of the retarding influence of packing. Obviously the packing factor cannot be eliminated in tubes even 600 mm. long and no advantage whatever is gained in employing tubes longer than 50 mm. On the contrary, there are many disadvantages.

SEDIMENTATION RATE OF ROULEAUX

The object in the present technique was to determine the rate of sedimentation of the rouleaux during the phase of settling at a time when the rouleaux are formed and settling at a uniform rate of speed, uninfluenced by the period of aggregation of the red blood cells into rouleaux on the one hand, or their packing in the bottom of the tube on the other. Unfortunately, it is impossible

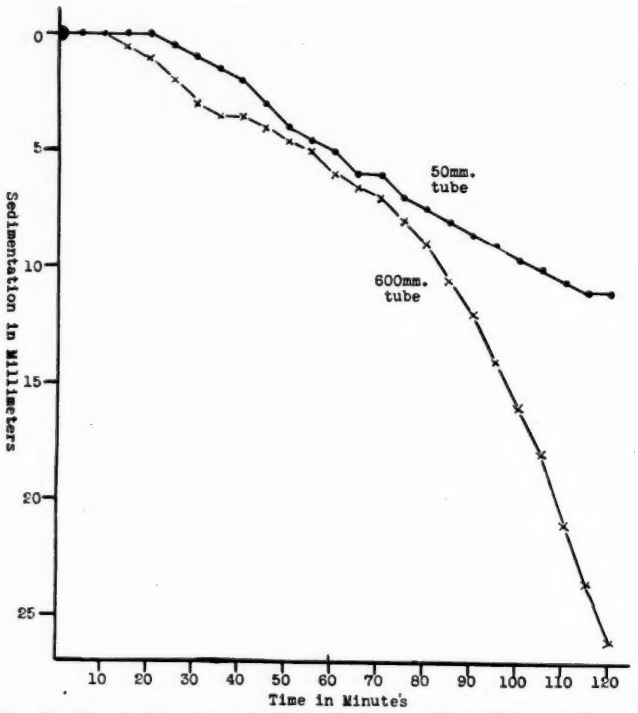

Fig. 5.—The length of tube is a decided factor in determining sedimentation rate, and this fact indicates the need for the adoption of a standard technique. The maximum drop in any five minutes in the 50 mm. tube is 1 mm.; in the 600 mm. tube, it is 2.5 mm. (same blood sample). The maximum rate in five minutes is reached much later in the 600 mm. tube than in the shorter 50 mm. tube.

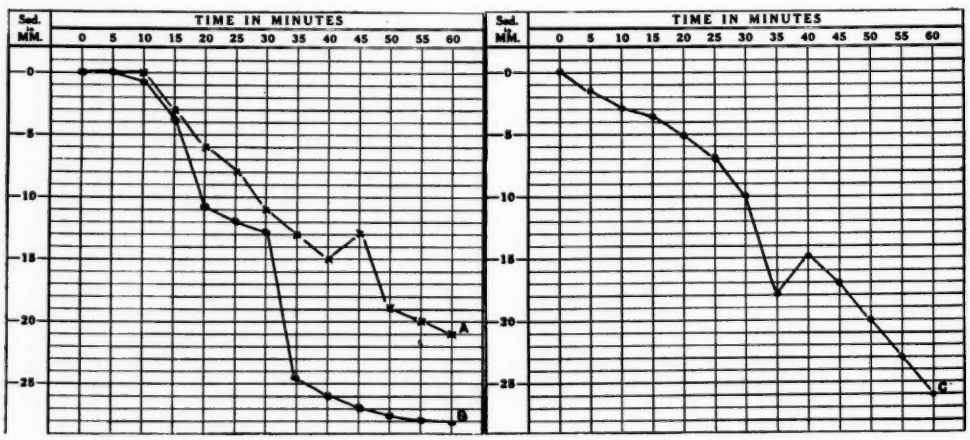

Fig. 6.—Bizarre sedimentation rate findings. Once rouleaux have formed, the rate of fall is fairly uniform. However, at times bizarre findings are observed, the explanation for which is not entirely clear, but they are in all probability mechanical in nature. It is worthy of note, however, that these bizarre records are self-correcting. Thus, if sedimentation is unduly slow in five minutes, the next reading will be unduly rapid (curve *B*), and vice versa (curve *C*). This again illustrates the unreliability of a single reading in determining sedimentation rate. The bizarre findings, while not common, were observed more frequently in tubes 100 and 200 mm. long than in 50 mm. tubes, and more commonly during the second half hour.

to eliminate the retarding influence of packing on sedimentation in rapidly settling blood in any tube of reasonable length, whether it be 50, 100, 200, or 600 mm. long (Fig. 4). In rapidly settling blood, the slowing effect of packing on sedimentation becomes evident well within the first hour, and the sedimentation rate is less in every one of the tubes mentioned than it would be normally in tubes of indefinite length. The only difference observed in tubes of 600 mm. or less in length was in the degree of slowing. It was also discovered that even in slowly settling blood, the rate of settling of the rouleaux, although slow in all tubes, is faster the longer the tube, and that the length of the tube is an important factor in determining sedimentation rate (Fig. 5). For practical reasons, therefore, it is necessary to determine the *maximum* sedimentation rate that the rouleaux can attain in a unit of time, e.g., five minutes, in a tube of fixed length. Such a unit would express the sedimentation velocity of the rouleaux at a time when it was *least* influenced by packing of the cells, and may be referred to as the maximum sedimentation rate or M.S.R., or simply sedimentation rate. Such a determination, for reasons mentioned above, would be independent of the anemia factor when present, and would be a true expression of sedimentation rate in that it denotes distance per unit of time and not distance at the end of a specified time as at present.

In the Cutler tube the maximum sedimentation rate in any five minutes can be determined during the first thirty minutes of settling, and no readings need be taken beyond that time, as the following study shows:

TABLE I

ANALYSIS OF 18 CASES (12.5% OUT OF A CONSECUTIVE SERIES OF 144) WITH SEDIMENTATION RATE FASTER IN A 5-MINUTE INTERVAL DURING THE SECOND 30 MINUTES OF SETTLING (CUTLER TUBE)

NO. OF CASES	M. S. R. IN ANY 5-MIN. PERIOD DURING FIRST 30 MIN. (MM.)	M. S. R. IN ANY 5-MIN. PERIOD DURING SECOND 30 MIN. (MM.)	DIFFERENCE (MM.)
3	0.5	1.0	0.5
2	1.0	1.5	0.5
1	2.0	2.5	0.5
1	0.5	1.5	1.0
3	1.0	2.0	1.0
2	2.0	3.0	1.0
1	3.5	4.5	1.0
1	2.0	3.5	1.5
1	5.0	7.0	2.0
1	2.0	4.5	2.5
1	4.0	7.0	3.0
1	2.5	6.0	3.5
18			

Only in the last three cases (2 per cent in this series) is the difference of some clinical significance.

Samples of blood from 144 consecutive patients were studied for their sedimentation rates by distributing parts of each sample into tubes 50, 100, and 200 mm. in length, respectively, and 5 mm. internal diameter. Readings were made every five minutes for one hour, and the results were recorded on sedimentation charts. (In an additional 93 cases simultaneous studies were made in 50 and 600 mm. tubes, respectively, but to simplify the presentation these are not discussed in detail.) The maximum settling in any five-minute period during the hour, after correcting for such bizarre findings as depicted in Fig. 6, was

determined for each sample of blood in each of the tubes studied. The results are recorded in Fig. 7. It will be noted that the M. S. R. made its appearance during the first thirty minutes of settling in the 50 mm. tube in 126 cases (87.5 per cent), in the 100 mm. tube in 104 cases (72.0 per cent), and in the 200 mm. tube in 81 cases (56.0 per cent). In the 600 mm. tube in many instances the M. S. R. did not make its appearance until the second hour of settling (Fig. 5).

Since a variation in the M. S. R. of 0.5 to 1 mm. is within normal limits,[3] a critical analysis of the 18 cases (12.5 per cent) in the Cutler series (50 mm. tube) with a maximum sedimentation rate in five minutes greater during the second thirty minutes of settling (Table I), discloses that in only three cases, the last three in the table, was the difference of some clinical significance, but not sufficiently great to discredit the findings in the first thirty minutes. Thus there is in this series a potential error, of little importance clinically, of approximately 2 per cent (3 cases in a consecutive series of 144) by limiting all readings to the first half hour when determining the M. S. R. This is a much lower percentage of error than with tubes of greater length, and is a distinct advantage in using the Cutler tube.

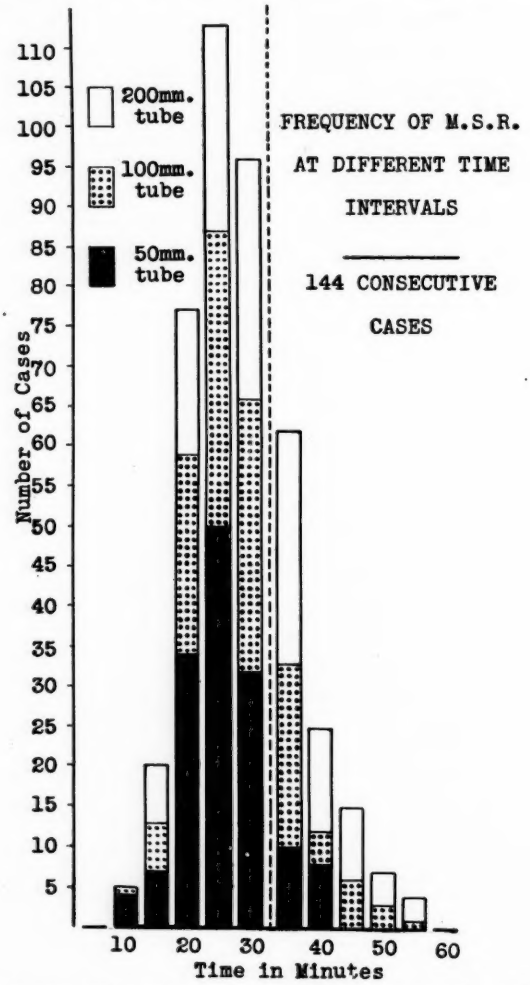

Fig. 7.—The maximum sedimentation rate (M.S.R.), expressed as the maximum settling in millimeters in any five minutes, was determined independently for each blood sample, in 50, 100, and 200 mm. tubes, respectively, and the frequency with which the M.S.R. occurred at different time intervals is recorded. In the 50 mm. tube the M.S.R. made its appearance in the first thirty minutes in 87.5 per cent of the blood samples studied (126 cases out of a consecutive 144). On the other hand, when the same bloods were studied in 100 mm. and 200 mm. tubes, the M.S.R. occurred in the first thirty minutes in only 72 and 56 per cent of cases, respectively. For details see text.

Other studies already completed[3] are additional proof that the Cutler tube of 50 mm. length and 5 mm. internal diameter will give sedimentation rate information with less chance of error and with greater convenience and efficiency, because such technical factors as position of tube, have less effect than in longer tubes. It would seem justifiable, therefore, to agree on a tube of this length in an effort to standardize the technique.

DETAILS OF TECHNIQUE

The essential features of the new technique are as follows:

Cutler sedimentation tubes of 1 c.c. capacity are used, graduated into 50 mm. divisions, with 0 at the 1 c.c. level. One-tenth of 1 c.c. of 3.8 per cent sodium citrate solution (age does not matter) is aspirated into a 2 c.c. syringe and blood, obtained by puncture of a suitable vein, drawn to the 1 c.c. mark (for convenience, larger quantities of blood and citrate, but in the same proportion, may be used). After drawing back the barrel of the syringe about 1 cm., the blood and citrate are gently mixed by tilting the syringe backward and forward several times. The contents are then emptied into the sedimentation tube up to the zero mark, and the tube is placed in a special rack. The position of the sedimenting column of erythrocytes is determined every five minutes for one-half hour. The observations are recorded on charts that have been designed for the purpose on which the horizontal lines represent the divisions on the tube and the vertical lines the intervals of time. Graphs are then constructed, which not only show the position of the sedimenting column of erythrocytes at any period of time during the first half hour, but also portray changes in velocity that occur during the process of sedimentation. This leaves a permanent, visual record, easily comprehended and readily compared. The biggest drop in any five-minute period during the first half hour is the maximum sedimentation rate in five minutes (M.S.R.) that the rouleaux will attain in the Cutler tube, and becomes the unit of comparison.*

EVALUATION OF SEDIMENTATION RATE

The evaluation of sedimentation rate presented in this paper, expressed as the maximum settling of the rouleaux in five minutes during the first thirty minutes (Cutler tube), is based on an analysis of 1,068 consecutive cases. In each instance sedimentation readings were made every five minutes for one hour, and graphs were constructed. At the same time the maximum sedimentation rate in any five-minute period during the first thirty minutes of settling (M.S.R.) was determined for each graph. The graph and maximum sedimentation rate were then compared and a tabulation was made (Table II). Cases with a maximum sedimentation rate of 2 mm. or less were restudied as a separate group, and a detailed comparison was made between the maximum sedimentation rate and the sedimentation index (Table III).

It has been generally accepted that in the original Cutler technique a graph represented by a horizontal line is a normal sedimentation finding and that all

*In large institutions where it is desirable to include the sedimentation rate in the routine blood determinations and heavy laboratory schedules make immediate examination impractical, reliable and accurate results will be obtained by distributing tubes containing 0.2 c.c. of 3.8 per cent sodium citrate solution and having 2 c.c. of blood syringed into this container at the time blood is drawn for other determinations. This amount suffices for use in the 1 c.c. Cutler tube (50 mm. by 5 mm. column). The test may be performed at any time within four hours of citration. The time interval clock for announcing five-minute intervals will be found time-saving and practical.

other graphs are pathologic findings of increasing clinical significance. Thus a diagonal line with a sedimentation index of 15 mm. or less, although pathologic, is usually associated with disease of slight intensity, as a rule without clinical manifestations of toxemia; a diagonal line with an index of 16 mm. or more with disease of slight to moderate intensity, but accompanied as a rule by constitutional symptoms of slight degree; a diagonal curve, with disease of moderate intensity accompanied usually by toxemia of moderate degree; and a vertical curve, with disease of marked intensity, associated almost invariably with marked constitutional symptoms. In the same technique the sedimentation index represents the total sedimentation at the end of one hour expressed in millimeters. A sedimentation index of 8 mm. or less is considered normal; an index of 9 mm. or more, pathologic.

With these explanatory remarks the following interpretation may be placed upon the maximum sedimentation rate in five minutes (M.S.R.).

TABLE II

EVALUATION OF MAXIMUM SEDIMENTATION RATE IN TERMS OF SEDIMENTATION GRAPH
(BASED ON 1,068 CONSECUTIVE CASES)

There is a distinct correlation between maximum sedimentation in five minutes and sedimentation graph. H.L., horizontal line; D.L.—15, diagonal line, sedimentation index 15 mm. or less; D.L.—16, diagonal line, sedimentation index 16 mm. or more; D.C., diagonal curve; V.C., vertical curve; M. S. R. is expressed in millimeters.

	SEDIMENTATION GRAPH					
MAXIMUM SEDIMENTATION RATE (M. S. R.)	·	H.L.	D.L.-15	D.L.-16	D.C.	V.C.
	NO. CASES	NO. CASES	NO. CASES	NO. CASES	NO. CASES	NO. CASES
0.5	158	156	2			
1.0[1,2]	164	143	21			
1.0[3-5]	89	13	76			
1.5	74	3	67	4		
2.0	125		79	46		
2.5	44		6	38		
3.0	103		2	95	6	
3.5	33			16	17	
4.0	81			39	42	
5.0	55				55	
6.0	44				44	
7.0	31				31	
8.0	22				22	
9.0	15				15	
10.0	12				10	2
11.0	8				1	7
12.0	5					5
13.0	1					1
14.0	1					1
15.0	1					1
16.0						
17.0	1					1
18.0						
19.0						
20.0	1					1

Normal Sedimentation Rate.—In Table III are analyzed 610 cases in this study with a maximum sedimentation rate of 2 mm. or less. These are subdivided into five groups: (1) those with an M.S.R. of 0.5 mm. or less; (2) an M.S.R. of 1 mm., but with the 1 mm. rate not repeated more than twice during the first thirty minutes of settling, expressed in the tables as M.S.R.[1-2]; (3) an M.S.R. of 1 mm., but with the 1 mm. rate repeated three or more times during the first

thirty minutes of settling, expressed in the tables as M.S.R.$^{3-5}$; (4) an M.S.R. of 1.5 mm.; and (5) an M.S.R. of 2 mm. Each maximum sedimentation rate is compared with the corresponding sedimentation index as defined above, and a table of parallel values is constructed.

It is apparent from a study of Table III that only a maximum settling in five minutes of 1 mm. or less, with the maximum rate of 1 mm. in five minutes not repeated more than twice during the first thirty minutes of settling, is normal, and that only such values correspond to a sedimentation index of 8 mm. or less in the original Cutler technique. When the maximum sedimentation rate in five minutes is 1 mm., but the rate is repeated three or more times during the first thirty minutes, the rate, as judged by the sedimentation index, is increased and therefore pathologic.*

TABLE III

EVALUATION OF MAXIMUM SEDIMENTATION RATE IN TERMS OF SEDIMENTATION INDEX

Only a maximum sedimentation rate in five minutes (M. S. R.) of 1 mm. or less with the 1 mm. rate not repeated more than twice during the half hour is normal. It would not be grossly incorrect, however, in order to simplify matters, to speak of a maximum settling in five minutes of 1 mm. or less as normal, and anything more as abnormal.

MAXIMUM SEDIMENTATION RATE

Sedimentation Index (Cutler)	0.5	1[2]	1[3-5]	1.5	2
	NO. CASES	NO. CASES	NO. CASES	NO. CASES	NO. CASES
Normal 1	16	2	86%—index of 9 or more	95% have index of 9 or more	All have index of 9 or more
Normal 2	41	10			
Normal 3	34	17			
Normal 4	16	19			
Normal 5	16	16	1		
Normal 6	21	26	2	1	
Normal 7	7	30	1	1	
Normal 8	5	23	9	1	
9	2	14	10	5	3
10	99% have index of 8 or less	6	25	11	2
11		87% have index of 8 or less	20	8	6
12			13	18	8
13			5	12	16
14			1	5	22
15			2	8	22
16			14% have index of 8 or less	1	15
17					8
18				1	10
19				1	8
20				1	5

Increased Sedimentation Rate.—Table II compares the sedimentation graph with the corresponding maximum sedimentation rate, and permits the following evaluation of an increased maximum sedimentation rate:

(1) A maximum sedimentation rate of 1 mm. (repeated three or more times), but not greater than 2 mm., corresponds to a diagonal line with a sedimentation index of 15 mm. or less, and represents disease of slight intensity, usually without constitutional symptoms. (2) M.S.R. of 2.5 to 4 mm. corresponds to a diagonal line with an index of 16 or more and represents disease of slight to moderate intensity, usually associated with some clinical manifestations of toxemia. (3) A M.S.R. of 5 to 9 mm. corresponds to a diagonal curve

*The number of times the maximum rate in five minutes repeats during the first thirty minutes of settling gives additional and detailed information on the rapidity of sedimentation—the more times it repeats the more rapid the settling. To simplify matters, although not strictly correct, one can consider a maximum settling in five minutes of 1 mm. or less as normal, and anything more as abnormal.

and represents disease of moderate intensity, usually associated with moderate constitutional symptoms. (4) A M.S.R. of 10 mm. or more corresponds to a vertical curve and represents disease of marked intensity characterized, as a rule, by marked constitutional symptoms.

This indicates the requirements for precise determination and interpretation of the sedimentation rate. The unit maximum sedimentation rate in five minutes (M.S.R.) needs no correction for anemia; it cannot be misunderstood for it is determined objectively. It is important to remember that the sedimentation rate of the rouleaux is different in tubes of different length. Should one desire to use a tube longer than 50 mm. the same principles hold true, but a different scale must be set up. The different scales, however, have a definite relationship to each other and can be readily compared. Thus, compared with the 50 mm. tube, the rate is one-third faster in the 100 mm. tube, twice as fast in the 200 mm. tube, and three to four times faster in the 600 mm. tube, a ratio of approximately one-third increase in sedimentation for every 50 mm. increase in the length of the tube.

SUMMARY

A new technique, a modification of the Cutler, is presented for determining erythrocyte sedimentation rate; it is based on the fundamental fact that it is the size of the rouleaux that determines the rate of settling. This technique is precise, simple, and time-saving. The results are easy to interpret and need no correction for anemia. The method embodies principles that are applicable to all tubes. The essential features are as follows:

Cutler sedimentation tubes of 1 c.c. caapcity are used, graduated into 50 mm. divisions, with 0 at the 1 c.c. level. One-tenth cubic centimeter of 3.8 per cent sodium citrate solution and 0.9 c.c. of blood obtained by puncture of a suitable vein are gently mixed in a 2 c.c. syringe and poured into the sedimentation tube, and the tube is placed in a special rack. The position of the sedimenting column of erythrocytes is determined every five minutes for one-half hour and is recorded on special charts. The maximum settling in any five minutes during the half hour is the sedimentation rate and becomes the unit of comparison.

For all practical purposes a maximum settling in five minutes of 1 mm. or less is normal. Everything else is abnormal and therefore pathologic. Generally speaking, maximum rates in five minutes between 1.5 and 4 mm. indicate disease of slight intensity; rates of 5 to 9 mm. indicate moderate intensity, and rates of 10 mm. or more, marked intensity.

Evidence is presented that the 1 c.c. Cutler tube is a practical tube for sedimentation rate determination, giving reliable and accurate results under average conditions. This tube appears suitable for standardization of technique.

The sedimentation charts may be obtained from Hartenstine Printing House, 206 DeKalb Street, Norristown, Pa.; the tubes, from Arthur H. Thomas Co., 230 S. 7th Street, Philadelphia, Pa.

REFERENCES

1. Cutler, J. W., Park, F. R., and Herr, B. S.: Influence of Anemia on Blood Sedimentation, Am. J. M. Sc. **195**: 734, 1938.
2. Cutler, J. W.: Graphic Presentation of the Blood Sedimentation Test, Am. J. M. Sc. **171**: 882, 1926.
3. Park, F. R., and Cutler, J. W.: Technical Factors Influencing Sedimentation Rate. In preparation.

RAPID DIAGNOSTIC METHOD FOR TESTING THE VIRULENCE OF CORYNEBACTERIA*

VERA B. DOLGOPOL, M.D., AND HELEN Y. MARKUS, M.T.
NEW YORK, N. Y.

VIRULENCE tests for the bacilli of the Corynebacterium group, carried out with pure cultures, require at least five to six days. While working with cultures of the Corynebacteria free from contaminating flora at the Laboratories of the Willard Parker Hospital, we succeeded in reducing to four days the time required for a report on the presence of toxin-producing diphtheria bacilli in swabs from the nose and throat. This was accomplished by culturing the material submitted for examination on a tellurite blood agar medium, and by using suspensions from pooled pickings of the Corynebacteria for virulence tests without subculturing them on other media.

Potassium tellurite, when incorporated in culture media in appropriate amounts, inhibits the growth of most pyogenic bacteria, without interfering with the growth of the bacilli of the Corynebacterium group (potassium tellurite media of Clauberg,[1] Pergola,[2] Anderson and co-workers,[3] and Horgan and Marshall[4]). The colonies of the Corynebacteria, grown on these media from a material rich in pyogenic flora, reach in eighteen hours a greater size than on other media where they grow among numerous colonies of contaminants. Suspensions made from several colonies picked from tellurite media are heavy enough to be used for virulence tests. The few contaminants that have overcome the inhibitory action of the potassium tellurite, usually the staphylococci, are readily identified by the appearance of their colonies and, in doubtful cases, by means of smears.

METHOD

Culture Medium.—The medium used was a slightly modified one of Horgan and Marshall. The tellurite solution was prepared by autoclaving 1 Gm. of potassium tellurite in 100 c.c. of distilled water. Eight cubic centimeters of this solution mixed with 5 c.c. of defibrinated horse blood was kept in the icebox for from one to three days and then added to 100 c.c. of melted 2 per cent agar beef heart medium pH 7.4 to 7.6, at 50° to 60° C. The medium was distributed in Petri dishes. The plates remain good for about three weeks if kept in the icebox.

As the solubility of the potassium tellurite varies from one batch to another, and some of it may remain undissolved after autoclaving, the presumably 1 per cent solution from a new supply of the chemical was tested with a known culture of the diphtheria bacillus. If, after the use of 8 c.c. of the solution in

*From the Pathologic Laboratories of the Willard Parker Hospital, New York.
Received for publication, December 21, 1939.

the medium, the colonies of the diphtheria bacillus were small in eighteen hours, 7.5 c.c. was used instead, and the growth then was found to be satisfactory.

Preparation of the Suspension.—Swabs from the nose and throat were streaked on plates of the tellurite medium. The next morning the cultures were examined with a hand lens. Smears were made from portions of several colonies of various morphology, and stained with the Ponder stain in order to avoid inclusion of the staphylococci in the suspension. No attempt was made to identify the three types of the diphtheria bacillus or to exclude the diphtheroids. However, colonies of various morphology were included in the suspension. Five or more colonies of the Corynebacteria, picked off the plate, were suspended in 2 or 3 c.c. of broth which assumed a light gray color. When tested in Hopkins' tubes, the suspensions proved to contain 0.15 per cent of bacillary bodies.

Virulence Test.—Two-tenths cubic centimeter of the suspension was inoculated intracutaneously in the shaved abdomen of a guinea pig; three to four tests were made on one animal. A similar amount was injected intraperitoneally into another guinea pig which was used for the same number of control tests, and 500 units of diphtheria antitoxin was given subcutaneously. The guinea pigs were examined in 24, 48, and 72 hours. A black or purple area on the site of inoculation in the unprotected animal indicated the presence of a toxin-producing bacillus in the suspension. No discoloration and no inflammation of the skin was ever observed in the control animals.

In order to establish whether the results of the rapid virulence test were comparable with those obtained by the usual methods, 55 duplicate tests were made with suspensions of the Corynebacterium colonies grown on tellurite medium and with cultures of those bacteria isolated from ordinary blood agar plates.

Duplicate swabs were obtained from the material to be examined. One swab was streaked on the tellurite medium, and the suspension from the colonies of the Corynebacteria for the virulence test was made the next day, as described in the method; the final report on the virulence was ready on the fourth day. A duplicate swab was streaked on a plate of ordinary 5 per cent blood agar. In eighteen hours the colonies of the Corynebacteria, growing among the contaminants, were small, and those not contiguous with the colonies of the contaminants could not supply enough material for a bacterial suspension sufficiently heavy for the virulence test. Subcultures on Loeffler slants had to be made from these colonies, and from twenty-four to forty-eight hours more elapsed before a sufficient growth was secured for the bacterial suspension. The guinea pig was thus inoculated two or three days after the material had been received for the examination, and the virulence test was completed on the fifth or sixth day, or one or two days later than from tellurite plates.

In 54 cases the results of the virulence tests with the two bacterial suspensions were identical (28 cultures of toxic diphtheria bacilli, 26 cultures of non-toxic bacilli). In one case in which only 3 colonies of the Corynebacteria were available after eighteen hours' incubation on the tellurite medium, the suspension showed no toxin production, while the subculture from the blood plate revealed

the presence of toxin-producing bacilli. No discrepancy was observed when five or more colonies were used for the preparation of the suspension. Apparently, if the diphtheria bacilli are at all present in the material inoculated, at least one colony of toxin-producing bacilli is likely to be included in a suspension from five colonies.

The importance of pooling picked colonies for a single virulence test in order to avoid false negative results is mentioned by Fraser and MacNab.[5]

The incorporation of the potassium tellurite in the culture media does not affect the production of the toxin by the diphtheria bacilli, according to Clauberg,[6] Zopoth,[7] and others.

No black discoloration of the guinea pig skin by the gray suspension of the tellurite-grown bacteria was ever observed in the antitoxin-protected animals. No discoloration other than toxic could, therefore, be expected in the nonprotected animals, and no false positive reactions were observed. However, we found it desirable to establish what amounts of the potassium tellurite might cause inflammation, necrosis, or discoloration of the skin. The use of 0.05 and 0.025 c.c. of a 1 per cent solution of potassium tellurite produced blackening of the site of intracutaneous injection; 0.015 c.c. produced a minute black dot, but smaller amounts left the skin unchanged. The amount of potassium tellurite in 0.2 c.c. of the bacterial suspensions used in the tests would be infinitely smaller than in 0.015 c.c. of a 1 per cent solution, so that no confusing discoloration of the guinea pig skin could be expected.

Suspensions of Corynebacteria grown on tellurite media were used occasionally for diagnostic virulence tests by other workers. Fraser and MacNab, in a review of the subject of virulence tests, mention in a brief paragraph the possibility of using these suspensions and indicate the need for pooling the suspensions from several colonies. Pavan,[8] in a clinical article on the importance of virulence tests in patients and carriers of "modified diphtheria bacilli" indicates that crude (i.e., whole) cultures from slants of Pergola's medium have been used for bacterial suspensions.

While the use of Corynebacteria grown on tellurite media for the virulence tests is not new, its application to a four-day diagnostic test seems to be worth mentioning.

Four-day diagnostic virulence tests have heretofore been performed with suspensions from the original Loeffler slant growths ("field cultures"), as recommended by Havens and Powell.[9] However, the presence of pyogenic contaminants in the washings from the crude cultures often produces a marked inflammation and suppuration at the site of intracutaneous inoculation, making the reading difficult.

Modified field cultures on Loeffler slants, with a diminished number of pyogenic contaminants, may be obtained by using subcultures from the rapid culture swabs of Brahdy, Lenarsky, Smith, and Gaffney.[10] Their use also permits a four-day report on the virulence. However, this method requires subculturing after four hours. While very useful in an individual case, it is inconvenient if cultures continue to arrive at the laboratory at various hours of the day. Besides, some pyogenic bacteria usually get into the subculture.

The method of using picked colonies of the Corynebacteria from tellurite blood plates for the preparation of the bacterial suspensions, as recommended here, has the advantage of rapidity attained by the use of field cultures, combined with freedom from pyogenic microorganisms. In addition, there is the saving of labor and media by abolishing the need for subculture.

SUMMARY

A practical four-day diagnostic test for the virulence of bacilli of the Corynebacterium group is suggested.

The bacterial suspensions for guinea pig inoculation are prepared from colonies of the Corynebacteria cultured from the nose and throat swabs on blood agar potassium tellurite plates.

No false positive reactions are observed.

False negative results are avoided by the use of five or more colonies for the preparation of the bacterial suspensions.

We wish to thank Dr. Ralph S. Muckenfuss, Director of the Bureau of Laboratories of the New York City Health Department, for his advice in the preparation of this report.

REFERENCES

1. Clauberg, K. W.: Ueber einen Elektivnährboden für Diphtheriebazillen, der eine makroskopische Plattendiagnostik ermöglicht, Zentralbl. f. Bakt., Abt. 1 Orig. **114**: 539, 1929.
   Clauberg, K. W.: Weitere Mitteilung zur makroskopischen Diphtheriebazillendiagnostik, Ibid. **120**: 324, 1931.
2. Pergola, M.: La difterite, in trattato italiano di igiene, Torino, 1930, Unione tip. edit. Torinese, Vol. 6, Fasc. 4, p. 42.
3. Anderson, J. S., Happold, F. C., McLeod, J. W., and Thomson, J. G.: On the Existence of Two Forms of Diphtheria Bacillus—B. diphtheriae gravis and B. diphtheriae mitis and a New Medium for Their Differentiation and for the Bacteriological Diagnosis of Diphtheria, J. Path. & Bact. **34**: 667, 1931.
4. Horgan, E. S., and Marshall, A.: A Simple Tellurite Medium for the Isolation of C. diphtheriae, J. Hyg. **32**: 544, 1932.
5. Fraser, D. T., and MacNab, A. L. (Committee): Intracutaneous Virulence Test for Corynebacterium diphtheriae, Am. J. Pub. Health **27**: 121 (Suppl., March), 1937.
6. Clauberg, K. W.: Ergänzende Ausführungen über Brauchbarkeit, Herstellung und Verwendung der neuen Blutwasser-Glyzerolat-Tellur-Nährplatte zur bakteriologischen Diphtheriediagnostic, Zentralbl. f. Bakt., Abt. 1 Orig. **128**: 153, 1933.
7. Zopoth, J.: La culture des bacilles diphthériques sur les millieux telluriques, Compt. rend. Soc. de biol. **121**: 785, 1936.
8. Pavan, G.: Bacilli difterici modificati e reazione intracutanea in cavia, Gior. di batteriol. e immunol. **20**: 1148, 1938.
9. Havens, L. C., and Powell, H. M.: The Use of the Original Diagnostic Culture for the Determination of the Virulence of Diphtheria Bacilli, Am. J. Hyg. **2**: 234, 1922.
10. Brahdy, M. B., Lenarsky, M., Smith, L. W., and Gaffney, C. A.: A Rapid Method for the Identification of Diphtheria Bacilli, J. A. M. A. **104**: 1881, 1935.

TECHNIQUE AND COMPARATIVE VALUES OF CROSS AGGLUTINATION METHODS*

A Study of Centrifuge, Slide, and Hanging-Drop Techniques

Norman W. Henry, M.D., Philadelphia, Pa.

THE cross-matching of recipient and donor bloods necessitates the use of some procedure which allows the mixing of cells and plasma (or serum) in such a way as to be read by the naked eye or microscope. For a long time the procedure in this hospital and in many others, the hanging-drop suspension was the method of choice. Of late we have used the thirty-minute open slide procedure with entirely satisfactory results.

In their article on "Intra-group Agglutinins in Blood Transfusions," Culbertson and Ratcliff compared the hanging-drop method with the rapid centrifuge method of Landsteiner and found the latter to be distinctly more sensitive. On communication with Ratcliff, we have learned that in using the hanging-drop method no specific effort was made to agitate the serum-cell mixture because this was technically impractical. We suspected from preliminary observations that the hanging-drop procedure was less sensitive because of the omission from the technique of some form of agitation. Shaking or some form of agitation is an essential part of any agglutination technique, the effect being apparently to throw particles or cells together mechanically, thus facilitating agglutination.

In order to prove this suspicion the following comparative studies on the centrifuge, hanging-drop, and open slide methods were carried out. Erythrocytes (II-A) from freshly drawn or bank blood, containing approximately 0.4 per cent sodium citrate, were washed three times in normal physiologic saline and a 5 per cent saline suspension was prepared. The titer of IV-0 plasma was then determined by each of the three methods.

The open slide method consists of thoroughly mixing a drop of cell suspension with several drops of plasma (or serum) on a ringed glass slide and allowing this to stand for thirty minutes under a Petri dish. A piece of moist filter paper is placed under the dish to prevent evaporation. The cells are thoroughly agitated by twirling and tilting the slide and the preparation is observed under the microscope.

The centrifuge method consists of shaking a few drops of plasma with one drop of washed cells for a minute and then incubating at 37° C. for five minutes. This is followed by centrifuging for three minutes and reading macroscopically. The hanging-drop method is similar to the open slide method,

*From the Laboratory of the Episcopal Hospital, Philadelphia.
Received for publication, January 9, 1940.

except that a hollow ground chamber and cover slip are used and the readings are made at the end of twenty minutes *without shaking.*

The sensitivity of the centrifuge method and open slide method was observed in the following procedure: Progressive geometric dilutions of plasma with saline were prepared (up to a dilution of 1:256) in 10 mm. test tubes. An equal size drop of the washed cell suspension was placed into all the tubes. Drops from each of these dilutions were placed on open glass slides, and the technique as described above followed. The remainder of the mixture in the test tubes was treated according to the centrifuge technique. This procedure enabled us to have the identical preparation in both set-ups. The results in Table I show that both methods were equally sensitive. Readings are microscopic.

TABLE I

PLASMA DILUTION	EXPERIMENTS 1		2		3		4		5	
	C.M.	SLIDE	C.M.	SLIDE	C.M.	SLIDE	C.M.	SLIDE	C.M.	SLIDE
0	4	4	4	4	4	4	4	4	4	4
2	4	4	4	4	4	4	4	4	4	4
4	4	4	4	4	4	4	3	4	4	4
8	3	3	3	4	2	2	3	3	4	4
16	2	2	2	2	1	1	3	2	4	3
32	1	2	2	2	1	1	0	1	3	2
64	1	1	1	1	0	1	0	1	3	1
128	0	1	1	1	1	0	0	0	1	0
256	0	0	0	0	0	0	0	0	0	0

The next procedure undertaken was to determine the comparative values with reference to the hanging-drop method. In all methods the cell plasma mixtures were identical for each dilution. Results are shown in Table II. Readings are microscopic.

TABLE II

PLASMA DILUTIONS	EXPERIMENT 1			EXPERIMENT 2		
	C.M.	SLIDE	H.D.	C.M.	SLIDE	H.D.
0	4	4	3	4	4	3
2	3	4	3	4	4	3
4	3	3	2	4	3	3
8	2	3	1	4	2	2
16	1	2	0	2	1	0
32	0	1	0	1	0	0
64	0	1	0	0	0	0
128	0	0	0	0	0	0
256	0	0	0	0	0	0

In comparing all three methods, one can readily see the close relationship of the centrifuge and the open slide methods, with some inferiority of the hanging-drop procedure. In preparing the cell-plasma mixture, the centrifuge method and the slide method permit shaking or agitation, a necessary requisite for any antigen antibody reaction. Both centrifuge and slide methods permit the application of this principle. With the hanging-drop preparation agitation is technically impractical, as it causes the suspension to flow to the line of glass contact, where observation is difficult. In several instances, agglutination was observed on the open slide method before and after agitating, and the titer was seen to rise two or three dilutions as a result of agitation.

We conclude, therefore, from our observations, that the centrifuge method and the open slide method have approximately the same degree of sensitivity, and that both are superior to the hanging-drop method. The centrifuge method has the advantage of requiring less time (ten minutes, as compared to the open slide method, forty minutes). The open slide procedure, however, facilitates the study of cold agglutination and pseudoagglutination.

REFERENCE

Culbertson, C. G., and Ratcliff, A. W.: Reaction Following Intra-Group Blood Transfusion, Am. J. M. Sc. 192: 471, 1936.

AN INEXPENSIVE CONSTANT TEMPERATURE PARAFFIN OVEN*

ROLAND E. MILLER, PH.D., AND ROBERT P. MOREHEAD, M.D.
WAKE FOREST, N. C.

DUE to the cost of constant temperature paraffin ovens some difficulty is experienced in obtaining a sufficient number for satisfactory operation of a technical laboratory. This difficulty has been met in our school by the construction of an inexpensive but efficient oven.

This oven consists of a wooden box, 28 inches long, 17 inches deep, and 7 inches high, which is fitted with a permanent pyramidal top tapering gradually to a height of 14 inches (Fig. 1). The oven was constructed from 1 inch pine wood and the upper pyramidal portion was lined with galvanized sheet metal to reflect the heat and to protect the wood. One side of the box is hinged at its junction with the pyramidal top so that it serves as a door, forming an opening into the oven which is approximately 26 by 7 inches.

The oven has no fixed bottom and when in use it is merely placed upon a piece of sheet tin on the table. A porcelain tray, 22½ by 12½ inches, serves as a container for the infiltrating work. This tray readily slides in and out of the oven and greatly simplifies embedding.

Heat is supplied by three 100 watt bulbs set in sockets at the top of the pyramid, and is controlled by a thermoregulator of simple construction. The thermostat consists of a piece of ordinary glass tubing, 30 cm. long and 5 mm. in diameter, on the end of which is blown a ball about 20 mm. in diameter. Approximately 50 mm. from the ball the tubing was bent to form a "U" tube, with a ball on one end and an opening on the other (Fig. 2). The tube was filled with mercury and then inverted. This procedure was repeated until the entire tube, except about 10 mm. at the open end, was filled. Then several drops of chloroform were added. The chloroform with a small air bubble was forced into the ball by inverting the tube. Following this, a sufficient amount of mercury was removed from the tube to lower the mercury level to about

*From the Wake Forest College, School of Medical Sciences, Wake Forest.
Received for publication, January 19, 1940.

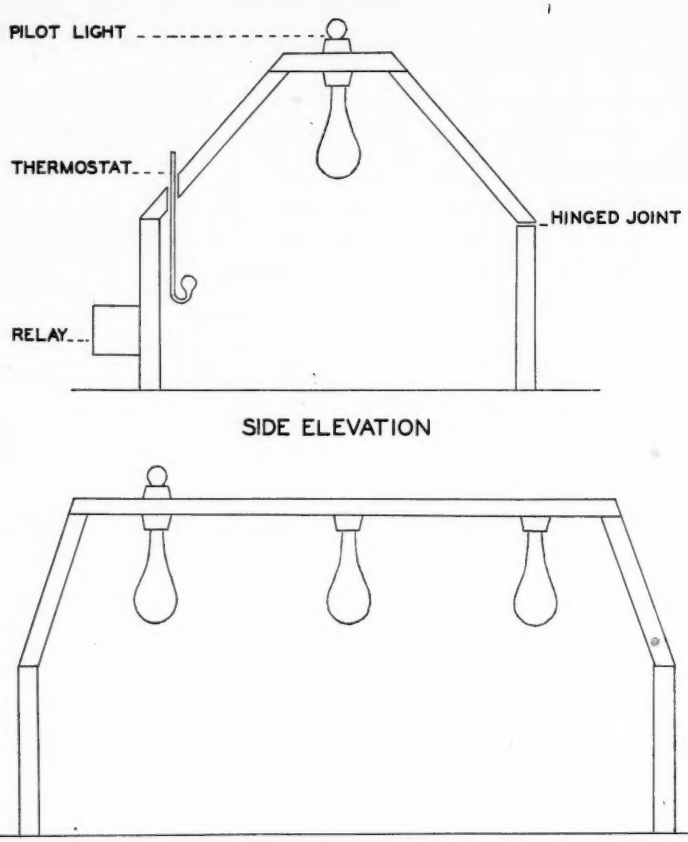

Fig. 1.—Front and side elevations showing the construction of the oven and the position of bulbs, thermostat, and relay.

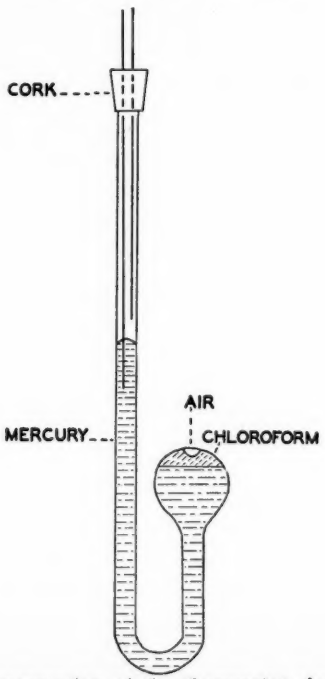

Fig. 2.—A diagrammatic representation of the thermostat showing details of construction.

100 mm. from the open end. Chloroform is added because its extreme volatility between the temperatures of 55° and 65° C. causes a large variation in the height of the mercury column and therefore greater sensitivity of the instrument. The tube is then placed in the oven with the long arm extending through a small hole in the top and is so adjusted that the ball is at the approximate level of the paraffin.

Two wires were forced through a small cork and suspended in the tube. One of the wires being longer than the other was passed below the surface of the mercury, while the other remained a few millimeters above the surface. These wires were attached to a Dunco relay which in turn regulated the current supplied to the light bulbs in the oven and to a small pilot light placed on the top of the oven. By raising or lowering the wire above the surface of the mercury, it is possible to control the temperature within the oven.

The advantage to this particular type of relay is that it operates on 110 volt current, but allows only a reduced current to pass through the thermostat, thereby eliminating the necessity of batteries or an additional transformer. Furthermore, this relay is desirable because it is so constructed as to eliminate the chatter which frequently accompanies the use of alternating current in such instruments.

The total cost involved in equipping this type of oven in our laboratories has been slightly less than $15.00 per oven. The cost of construction of the oven, including the wiring, was $3.90, while the relay cost $11.00. In addition to the low cost of construction the size of the oven allows a greater amount of usable space than most ovens of the same cost which generally lack the advantages of automatic temperature control.

Several of these ovens have been in use in our pathology and histology laboratories for the past year and have been found to be very satisfactory. The time required to bring the temperature in the oven to that of the melting point of paraffin, using three 100 watt bulbs, has been found to be about twenty minutes. With the door of the oven closed, the temperature, once obtained, will remain within one degree for an indefinite period.

It is suggested that following two months of continuous use the surface of the mercury should be cleaned in order to maintain the sensitivity of the thermostat. This may be easily done with a bit of cotton on the end of a small wire.

ANTIGEN PIPETTE FOR KLINE SYPHILITIC TEST*

EDWIN S. POWELL, COLUMBIA, S. C.

A STRONG and dependable pipette for use in dropping antigen in the Kline syphilitic test may easily be made from a 1 c.c. serologic pipette, 26 gauge syringe needle, rubber medicine dropper bulb, and cement (''Dart Household and General Purpose Cement'' has proved most satisfactory to me).

Select a fairly stout 1 c.c. pipette—cut off a small portion of the tip so that it will fit tight in the cup of the needle. Then cut off the top of the pipette at the 0.3 c.c. mark. By cutting down the length the pipette is easier to handle. Cut off the point of the needle about 1 mm. from the joint at the bottom of the cup by notching with a triangular file and breaking it off evenly.

Place a drop of cement on the tip of the pipette and push the needle on gently and firmly. With an applicator or match stem concentrate the cement evenly around the top of the needle so as to assure permanency and no leakage. Allow this to dry thoroughly. Put the rubber bulb on the top of pipette and it is ready for use. Should there be a loose fit between the pipette and the bulb, wrap with a rubber band.

This pipette delivers approximately 62 drops per 0.5 c.c., which is the size drop Kline recommends. Because of its speed it will be found especially useful in running large numbers of tests.

*From the Hygienic Laboratory, South Carolina State Board of Health, Columbia.
Received for publication, January 25, 1940.

A CONVENIENT TURNTABLE FOR STAINING JARS

A. BYRON LEONARD, PH.D., AND ALICE E. LEONARD, M.A.
LAWRENCE, KAN.

WHEN tissue sections are to be carried through an extensive series of reagents, a convenient arrangement of the containers is often difficult. The jars containing the reagents are often arranged in a long row, or in a series of shorter ones, but in any case the technician does not find all the jars within easy reach. However, by placing the staining jars on a turntable, such as the simple and inexpensive device described below, all the containers in a series become equally accessible to the operator.

Fig. 1 represents a sectional view of the device. In this illustration *C* is a circular piece of wood, 1½ inches in thickness and 24 inches in diameter, which comprises the platform of the turntable. In the center of the ventral side of *C* a circular depression, 1 inch deep and 6 inches in diameter, is made to receive *A*, which is a pinion obtained from the transmission gears of an old

Received for publication, January 17, 1940.

automobile. The pinion *A* has a collar on its ventral side which rests within the thrust bearing *B*. The bearing *B* is held in place by being inset into the block *D*, which in turn may be secured to the top of a table. Since the bearing contains a ball race and ball bearings, the platform *C* turns very easily, even when bearing a load of Coplin jars. The cost of materials for this very convenient device was slightly more than $1.00.

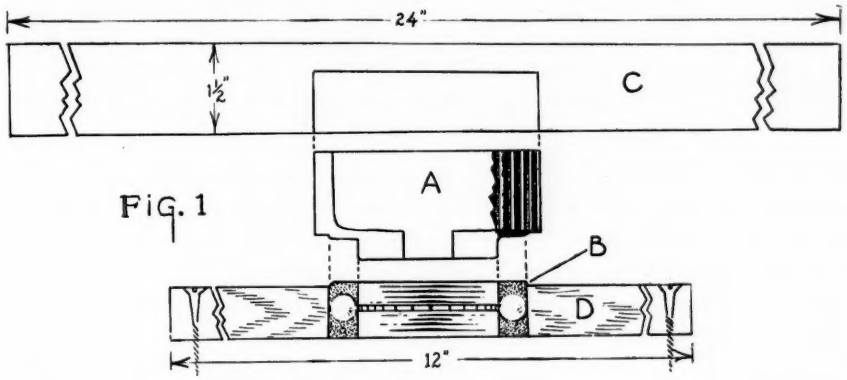

Fig. 1

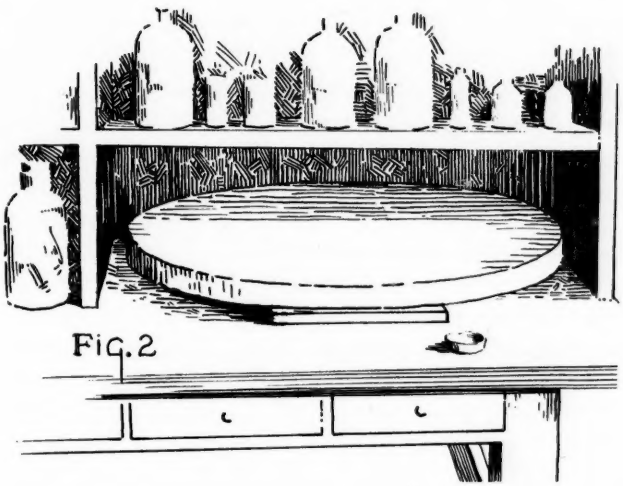

Fig. 2

Fig. 2 is a perspective view of the turntable. Upon the periphery of the platform may be placed from 20 to 22 Coplin staining jars, depending upon the size of the bases. The unoccupied space in the center of the platform makes a convenient place to put the tops of the jars when the latter are in use.

CHEMICAL

A RAPID METHOD FOR DETERMINING SPECIFIC GRAVITY OF BODY FLUIDS BY THE FALLING DROP PRINCIPLE*

CARROLL J. BELLIS, M.D., MINNEAPOLIS, MINN.

CHANGES in the density of whole blood or plasma are important in defining and recognizing the onset of shock and in determining the degree of hydremia. The well-known Hammerschlag method and its several modifications (Roy, Van Walsem, Exton) are time-consuming and elaborate. Gravimetry has the same disadvantages. Since the introduction by Barbour and Hamilton[1-3] of the falling drop method, the determination of blood specific gravity has become an acceptable routine clinical procedure. With this technique, the blood or plasma specific gravity may be determined in one or two minutes, using only a drop of the sample (0.01 c.c.), within a maximum error of ±0.0001 sp. gr. Guthrie[4] and Kagan[5] have employed the falling drop principle with an accuracy far greater than clinical experience necessitates.

In the surgical wards of the University of Minnesota hospitals we have employed the falling drop method to determine the state of hydration of patients and the plasma protein concentration. After Moore and Van Slyke[6] had formulated an expression whereby the protein concentration could be calculated if the plasma specific gravity were known, Nugent and Towle[7] discovered that the albumin-globulin ratio had no effect on specific gravity, since albumin and globulin exerted the same effect. Weech, Reeves, and Goettsch[12] then published formulas for accurately calculating the serum or plasma protein concentration from the specific gravity. Peters and Van Slyke[8] have indicated that of the methods for determining serum protein, the specific gravity method is sufficiently accurate. Spencer,[10] after making many comparative determinations, concluded that the falling drop method was more accurate than the weight method for determining serum protein. Similarly, the later work of Shuman and Jeghers[9] showed that detailed chemical procedures are no more accurate for routine work than the falling drop method. This communication presents a method of simplifying and accelerating the determination of blood or plasma specific gravity with the Barbour and Hamilton technique.

METHOD

From the Barbour and Hamilton alignment chart, a logarithmic progression was derived, and from an expression which designates the points on their chart, Table I was obtained. Much time is saved by using this table to obtain the apparent density differences.

*From the Department of Surgery, University of Minnesota Medical School, Minneapolis. Received for publication, August 8, 1940.

TABLE I

APPARENT DENSITY DIFFERENCES FOR VARIOUS DEGREES CENTIGRADE AND FALLING TIMES

TIME (SEC.)	20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	31°	32°	33°	34°
10	036	037	038	038	039	040	040	041	042	042	043	043	044	044	045
11	032	032	033	033	034	035	035	036	037	037	038	038	040	040	041
12	028	029	029	030	030	031	032	033	034	034	034	035	036	036	037
13	025	026	027	027	028	028	029	030	031	031	032	032	033	033	034
14	023	023	024	024	025	026	026	027	028	028	029	030	031	031	032
15	021	021	022	022	023	024	024	025	026	026	027	028	029	029	029
16	019	020	020	021	021	022	022	023	025	025	025	026	027	027	028
17	018	018	019	019	020	021	021	022	023	023	024	024	025	026	027
18	016	017	017	018	019	019	020	021	022	022	023	023	024	024	025
19	015	015	016	017	018	018	019	020	021	021	022	022	023	023	024
20	014	014	015	016	017	017	018	019	020	020	021	021	022	023	023
21	013	014	014	015	016	016	017	018	019	019	020	020	021	022	022
22	012	013	013	014	015	016	016	017	018	018	019	020	020	021	022
23	011	012	013	013	014	015	015	017	017	018	018	019	020	020	021
24	010	011	012	013	013	015	015	016	017	017	018	018	019	020	020
25	010	011	012	012	013	014	014	016	016	016	017	018	019	019	020
26	010	010	011	012	012	013	014	015	016	016	017	017	018	019	019
27	009	010	010	011	012	013	013	014	015	015	016	017	018	018	019
28	009	009	010	011	011	012	013	014	015	015	016	016	017	018	019
29	008	009	010	010	011	012	012	014	014	015	015	016	017	018	018
30	008	008	009	010	011	011	012	013	014	014	015	016	016	017	018
31	007	008	009	010	010	011	012	013	013	014	015	015	016	017	018
32	007	008	009	009	010	011	011	013	013	014	014	015	016	017	017
33	007	008	008	009	010	010	011	012	013	013	014	015	016	016	017
34	007	007	008	009	009	010	011	012	012	013	014	015	015	016	017
35	006	007	008	008	009	010	011	012	012	013	014	014	015	016	017
36	006	007	007	008	009	010	011	012	012	013	013	014	015	016	016
37	006	007	007	008	009	009	010	011	012	012	013	014	015	015	016
38	006	006	007	008	008	009	010	011	011	012	013	014	014	015	016
39	005	006	007	008	008	009	010	011	011	012	013	013	014	015	016
40	005	006	007	007	008	009	010	011	011	012	013	013	014	015	016
41	005	006	007	007	008	009	009	010	011	012	012	013	014	015	015
42	005	006	006	007	008	009	009	010	011	012	012	013	014	015	015
43	005	005	006	007	008	008	009	010	011	011	012	013	014	014	015
44	005	005	006	007	008	008	009	010	010	011	012	013	014	014	015
45	004	005	006	007	007	008	009	010	010	011	012	013	013	014	015
46	004	005	006	006	007	008	009	010	010	011	012	013	013	014	015
47	004	005	006	006	007	008	009	010	010	011	012	012	013	014	015
48	004	005	006	006	007	008	008	009	010	011	012	012	013	014	015
49	004	005	005	006	007	008	008	009	010	011	011	012	013	014	014
50	004	005	005	006	007	007	008	009	010	011	011	012	013	014	014
51	004	004	005	006	007	007	008	009	010	010	011	012	013	014	014
52	004	004	005	006	007	007	008	009	010	010	011	012	013	013	014
53	003	004	005	006	006	007	008	009	010	010	011	012	013	013	014
54	003	004	005	006	006	007	008	009	009	010	011	012	012	013	014
55	003	004	005	006	006	007	008	009	009	010	011	012	012	013	014
56	003	004	005	005	006	007	008	008	009	010	011	012	012	013	014
57	003	004	005	005	006	007	008	008	009	010	011	011	012	013	014
58	003	004	005	005	006	007	008	008	009	010	011	011	012	013	014
59	003	004	005	005	006	007	008	008	009	010	011	011	012	013	014
60	003	004	004	005	006	007	007	008	009	010	011	011	012	013	014
61	003	004	004	005	006	007	007	008	009	010	010	011	012	013	014
62	003	004	004	005	006	007	007	008	009	010	010	011	012	013	014
63	003	004	004	005	006	007	007	008	009	010	010	011	012	013	013
64	003	003	004	005	006	006	007	008	009	010	010	011	012	013	013
65	003	003	004	005	006	006	007	008	009	009	010	011	012	013	013
66	003	003	004	005	006	006	007	008	009	009	010	011	012	013	013
67	003	003	004	005	006	006	007	008	009	009	010	011	012	012	013
68	002	003	004	005	006	006	007	008	009	009	010	011	012	012	013
69	002	003	004	005	005	006	007	008	009	009	010	011	012	012	013
70	002	003	004	005	005	006	007	008	008	009	010	011	012	012	013

For most samples of plasma we use the 75.3 : 24.7 :: m-xylene : bromobenzene mixture which these investigators suggested. For more hydremic or hypoproteinemic plasma the 76.9 : 23.1 mixture is used; it has a specific gravity of 1.013. If the specific gravity of whole blood is to be determined, the 72.1 : 27.9 mixture is used; this mixture has a specific gravity of 1.043. As will be recalled, the falling times of exactly 0.01 c.c. of standard solution of potassium sulfate and sample through 30 cm. of m-xylene-bromobenzene mixture are determined with the stop watch, and the room temperature is noted. Table I gives the apparent density difference of each falling drop, the apparent density difference of the standard solution being given a – sign, that of the sample a + sign. The algebraic sum of these two (actual density difference) is then algebraically added to the specific gravity of the standard solution to obtain the specific gravity of the sample. Table II (made from the formula of Weech, Reeves, and Goettsch) gives the protein concentration of the serum corresponding to the specific gravities. If plasma is used, reference to the same table may be made for clinical purposes, since the error thus produced is less than 0.5 Gm. per 100 c.c.

TABLE II

SERUM PROTEIN CONCENTRATIONS CORRESPONDING TO SPECIFIC GRAVITY

SP. GR.	PROTEIN	SP. GR.	PROTEIN	SP. GR.	PROTEIN
1.017	3.37	1.025	6.16	1.033	8.94
1.018	3.72	1.026	6.51	1.034	9.29
1.019	4.07	1.027	6.85	1.035	9.64
1.020	4.42	1.028	7.20	1.036	9.98
1.021	4.77	1.029	7.55	1.037	10.33
1.022	5.11	1.030	7.90	1.038	10.68
1.023	5.46	1.031	8.25	1.039	11.03
1.024	5.81	1.032	8.59	1.040	11.38

Example:

Temperature 22° C.
Falling time of serum = 30 sec.
Apparent density difference +0.009 (Table I).
Falling time of standard (sp. gr. 1.035) = 20 sec.
Apparent density difference –0.015 (Table I).
0.009 – 0.015 = –0.006 (actual density difference).
1.035 – 0.006 = 1.029 = sp. gr. of serum.
1.029 is equivalent to protein concentration 7.55 per cent (Table II).

COMMENT

To date, the improvement described, namely, the already calculated chart of apparent density differences, has made possible the determination of blood or serum specific gravity in a few moments. The constant checks of this method of obtaining plasma or serum protein concentration with micro-Kjeldahl readings have been both gratifying and reassuring, and it is my feeling that this method of estimating total plasma protein concentration is fully as accurate as the well-known Kjeldahl method.

The fluid requirement of the surgical patient has ceased to be an arbitrary value or a matter of "hit or miss." Closely correlated with the sodium chloride

intake and excretion is the water balance, and an index of this may be obtained by plasma density determinations. Thompson, Ravdin, and Frank[11] have significantly commented on the effects of hypoproteinemia on wound disruption, a relation which may be due to edema or to the general lack of essential elements which accompany low protein intake. Preoperative preparation of patients for extensive surgical procedures can be assisted by rapid protein determinations with this method. During the critical postoperative period similar determinations will indicate the general state of the patient.

SUMMARY

A successful experience with the Barbour and Hamilton falling drop method for determination of plasma, serum, or whole blood specific gravity is reported. A table is presented for use in the rapid determination of the specific gravity, and a second table is given for obtaining the serum protein concentration when the specific gravity is known. The advantages of these are mentioned, and some indications for their employment are cited.

REFERENCES

1. Barbour, H. G., and Hamilton, W. F.: Blood Specific Gravity: Its Significance and a New Method for Its Determination, Am. J. Physiol. **69:** 654, 1924.
2. Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity, J. Biol. Chem. **69:** 625, 1926.
3. Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity. Some Clinical Applications, J. A. M. A. **88:** 91, 1927.
4. Guthrie, C. C.: An Apparatus for Quickly Measuring the Specific Gravity of Body Fluids, J. LAB. & CLIN. MED. **17:** 1158, 1932.
5. Kagan, B. M.: A Simple Method for the Estimation of Total Protein Content of Plasma and Serum. I. A Falling Drop Method for the Determination of Specific Gravity, J. Clin. Investigation **17:** 369, 1938.
   II. The Estimation of Total Protein Content of Human Plasma and Serum by the Use of the Falling Drop Method, J. Clin. Investigation **17:** 373, 1938.
6. Moore, N. S., and Van Slyke, D. D.: The Relationships Between Plasma Specific Gravity, Plasma Protein Content, and Edema in Nephritis, J. Clin. Investigation **8:** 337, 1930.
7. Nugent, R. L., and Towle, L. W.: The Specific Gravity of Synthetic Solutions of Serum Albumin and Serum Globulin, J. Biol. Chem. **104:** 395, 1934.
8. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Vol. II. Methods, Baltimore, 1932, Williams & Wilkins Co.
9. Shuman, H. H., and Jeghers, H.: The Value of Routine Blood-Protein Determinations, New England J. Med. **222:** 335, 1940.
10. Spencer, H.: The Water Content of Blood Serum. A Comparison of the Determination of the Specific Gravity by the Falling Drop Method and by Several Other Methods, Am. J. Dis. Child. **37:** 546, 1929.
11. Thompson, W. D., Ravdin, I. S., and Frank, I. L.: Effects of Hypoproteinemia on Wound Disruption, Arch. Surg. **36:** 500, 1938.
12. Weech, A. A., Reeves, E. B., and Goettsch, E.: The Relationship Between Specific Gravity and Protein Content in Plasma, Serum, and Transudate From Dogs, J. Biol. Chem. **113:** 167, 1935.

A SIMPLIFIED SYSTEM OF BUFFERS AND INDICATORS WITH OR WITHOUT GLASS ELECTRODES*

J. F. McClendon, Philadelphia, Pa.

BECAUSE a multiplicity of technical methods are demanded in medical laboratories, there is a need for saving the time required for mathematical calculations. For this reason, I built a potentiometer reading directly in pH.[6] A newer development is the direct reading glass electrode apparatus that eliminates all calculation in determining pH; however, it must be standardized frequently by a standard buffer because, if the proper buffer is not used, all the results will be incorrect. The following method eliminates all complicated mathematics and all errors caused by moisture in "dry" chemicals and by inaccurate volumetric apparatus used in the preparation of standard buffers. Furthermore, with a burette of uniform bore, buffers of graded series may be prepared for determining pH with indicators.

With the realization that Sörensen's values of pH were incorrect, physical chemists eliminated this criticism by coining the term "activity." Now pH is redefined as the logarithm of the reciprocal of hydrogen-ion activity. If one adds 0.04 to Sörensen's value, the correct result is obtained.

It has been shown that the ionic strength† of a solution affects the ionization of both indicators and buffers (salt error). But determinations of ionic strength may be avoided through the use of buffers and indicators that are affected very little by changes in ionic strength. Also calculations may be eliminated by the use of special graph paper.‡

An approximation should be made when these solutions are prepared. Otherwise difficulties may arise if solubilities or safe dilutions are exceeded. However, quantitative accuracy is not necessary except where indicated.

Indicators will act as buffers if they are at high concentration; hence, if they are to serve as indicators, they should be in very low concentration. Because of the low solubility of some indicators it has been customary to make their solutions 0.01 to 0.04 per cent. For uniformity of practice it is desirable to make the more soluble ones of the same concentration. Since dry indicators may not always wet with water and are more soluble in alcohol and since alcohol checks mold growth, it is well to weigh out 0.04 Gm. of the indicator, add 50 c.c. of alcohol to dissolve it (heat may be necessary), and then add 50 c.c. of water (some indicators will not completely dissolve until after the addition of water).

Methyl orange is very little, if at all, affected by ionic strength. Its "absorption" spectrum is given in Fig. 1 for both acid and alkaline solution. When light of wave length 5600 A. U. was used, the "absorption" (logarithm of

*From the Research Laboratory of Physiology, Hahnemann Medical College, Philadelphia. Aided by a grant from the American Philosophical Society.
Received for publication, December 8, 1939.

†Ionic strength = ½ (sum of concentration of each ion × its valence squared).

‡For sale at Hahnemann Medical College Book Shop, 235 N. 15th Street, Philadelphia.

the reciprocal of the transmittance) of the acid (un-ionized*) dye was 0.62, and the absorption of the alkaline (ionized) dye was 0.05; therefore the relative amount of the alkaline (ionized) dye may be determined. At pH 3.6 the absorption was 0.335; hence 50 per cent was ionized (0.62 + 0.05 divided by 2

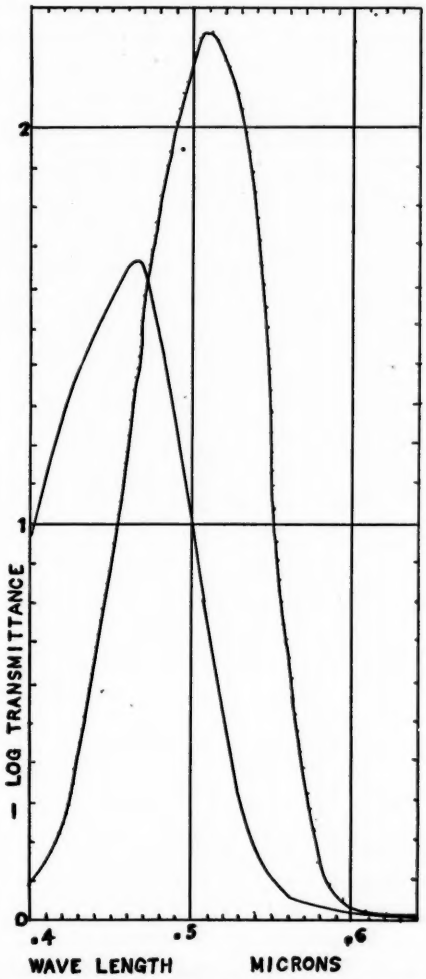

Fig. 1.—Absorption curves at 25° C. of 1 c.c. of 0.04 per cent methyl orange in 25 c.c. of N/10 HCl (tall rough curve) and N/100 NaOH (short smooth curve) made with a Coleman spectrophotometer. The ionization constant was computed from the absorption at 5600 A. U. (0.56 μ), and pH 1, 12, and 3.6 were determined with a Coleman glass electrode. The part above 2 is not graduated since this method becomes less accurate as complete absorption is approached.

equals 0.335). Fig. 2 shows on the left the amount of yellow (a) in the dye at different pH values from 1.6 to 5.6. (The red component of the dye is represented by $1 - a$ on the right.) A similar graph for phenol red† is also shown in Fig. 2, where red component is represented by a.

*It is usually represented as a "zwitterion" or amphoteric ion, but this may be considered un-ionized since the hydrogen ion that separated at one point attached itself at another.

†The salt correction for phthaleins (Kolthoff[5]) is related to buffer solutions of ionic strength equal to 0.1.

Ionic strength	Correction
0.50	−0.20
0.05	+0.11
0.01	+0.13
0.005	+0.15
0.0025	+0.16

Phenolphthalein behaves as a dibasic acid,* and its dissociation graph appears to be a composite of the two graphs in Fig. 2. A drop of phenolphthalein solution is used in the preparation of the salt component of the buffers, but this will not interfere with the use of other indicators at pH values less than 8. Similar graphs have been prepared for a number of other indicators.[7-9, 11, 12]

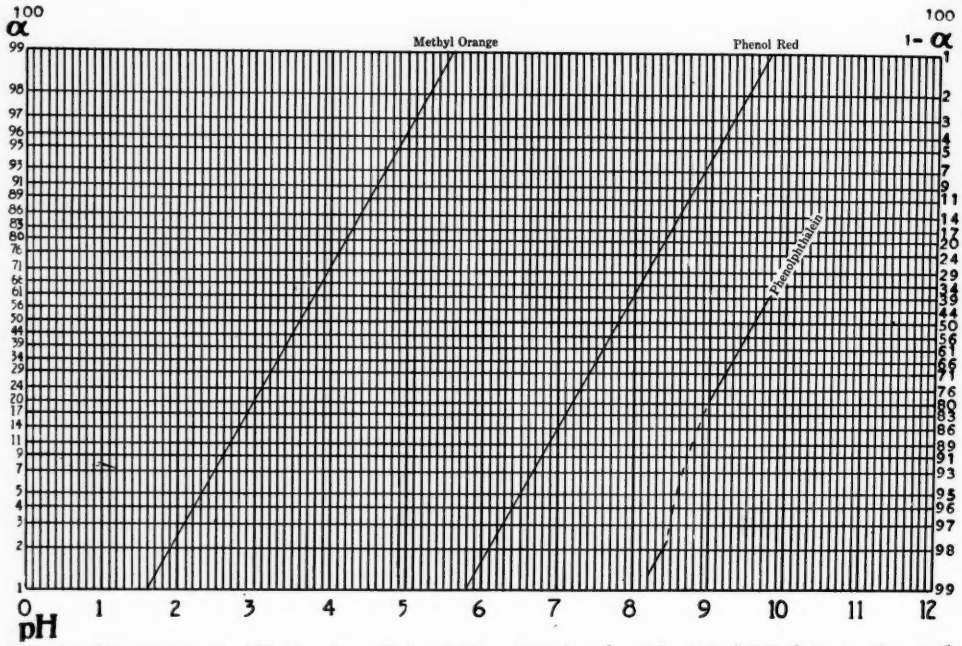

Fig. 2.—Percentage at 25° C. of methyl orange, phenol red, and phenolphthalein in the acid form (100α, left) and alkaline form [100 (1-α), right].

STOCK SOLUTIONS

Normal hydrochloric acid: Place 10 c.c. of concentrated HCl (approximately 38 per cent by weight, 45 per cent by volume, sp. gr. 1.19) in a 100 c.c. volumetric flask and dilute to 100 c.c. Transfer to a 200 c.c. glass-stoppered bottle and add 23 c.c. of water.†

Carbon dioxide-free water: Place 700 c.c. of distilled water in a round bottom, liter pyrex flask. Cover with a watch glass and boil vigorously for five minutes. Insert an oversized rubber stopper and cool under tap. Allow to remain stoppered until used.

Normal sodium hydroxide: Place 75 Gm. of NaOH and 75 c.c. of water in a pyrex flask, 200 c.c. or larger. Rotate until dissolved. Transfer the hot solution to a 100 c.c. pyrex flask. Place on the surface of the liquid a lump of paraffin, which will melt and exclude air. Wrap the flask in a towel to delay cooling and let it stand until the next day when the carbonate should have settled, at least from the top layer. Lift the solidified paraffin and insert the

*Michaelis and Gyemant[13] speak of three dissociation constants, but Kober and Marshall[4] found the trisodium salt to be colorless. Rosenstein[14] observed two dissociation constants.

†For more accurate N/10 HCl place the 1:1 acid in a still, distill off three-fourths, collect 25 c.c., and read barometer. At 760 mm. weigh out 18.017 Gm.; at 750 mm., 17.996 Gm.; at 740 mm., 17.975 Gm.; at 730 mm., 17.953 Gm.: Then dissolve and dilute to 100 c.c. (Hulett and Bonner[2]).

mouth end of a 10 c.c. pipette and suck up clear solution until the bulb is filled (reversal merely saves time). Run the solution into a 100 c.c. volumetric flask and fill to the mark with carbon dioxide-free water. If the flask will hold 10 c.c. more, run in 10 c.c. carbon dioxide-free water. This may be done with the same pipette, preferably without rinsing, by using a rubber tube to protect the mouth from sodium hydroxide and then sucking the solution up as far as the rubber tube to rinse the stem. Mix by inversion and rinse the pipette by sucking up several times; then transfer 10 c.c. to a small beaker or Erlenmeyer flask for titration with hydrochloric acid. Use one drop of phenolphthalein solution as indicator, and titrate to the end point, the first pink coloration that remains twenty seconds after stirring. Record the titer as 10+x hydrochloric acid and add 10x carbon dioxide-free water to the 100 c.c. of sodium hydroxide on transferring to a paraffin-lined bottle. The paraffin lining is made by pouring about 20 Gm. of melted paraffin (preferably with melting point over 60° C.) into a dry 200 c.c. cylindrical, glass-stoppered bottle and rolling it on the table while cooling (having dipped the dry stopper in melted paraffin to prevent sticking with sodium hydroxide).

Normal acetic acid: Mix 6 c.c. of glacial acetic acid with 100 c.c. of water.

Normal chloracetic acid: Weigh out 95 Gm. of monochloracetic acid (free from acetic acid, dichlor and trichlor), and place it in a 100 c.c. volumetric flask. Add water to dissolve the acid, and fill the flask to the mark.

Normal cacodylic acid: Weigh out 13.8 Gm. of cacodylic acid, and place it in a 100 c.c. volumetric flask. Add water to dissolve the acid, and fill the flask to the mark.

CONCENTRATED BUFFERS FOR STANDARDIZING GLASS ELECTRODES

Acetic-acetate buffer: Using the same pipette so that correction for error will not be necessary, place 25 c.c. of normal acetic acid in a 100 c.c. glass-stoppered bottle and 25 c.c. in a small beaker or Erlenmeyer flask, and add one drop of phenolphthalein solution. Fill a burette with normal sodium hydroxide and run in 20 c.c.; then very carefully titrate to the first pink coloration that stays after stirring for twenty seconds. Transfer this neutralized solution (sodium acetate) to the bottle, using very small amounts of carbon dioxide-free water to rinse the titration vessel. Pour rinse water into the bottle. The pH of this 50/50 mixture of acetic acid and its sodium salt will be 4.74 at 25° C. and will remain constant on dilution up to ten times or more with carbon dioxide-free water.

Chloracetic-chloracetate buffer: A 50/50 mixture of chloracetic acid and sodium chloracetate may be made in a similar manner. The pH of this solution will be 2.86 at 25° C. (Armstrong[1] found 2.8.)

Cacodylic-cacodylate buffer: A 50/50 mixture of cacodylic acid and sodium cacodylate may be made in a similar manner except that the titration should not be stopped at a pale pink but continued to maximum color. The pH of the 50/50 mixture will be 6.2 at 25° C.

Borax buffer: A solution of borax may have a pH of 9.15 to 9.2, but its pH is influenced by the ionic strength and therefore is changed by dilution. Its advantage lies in the fact that no titration is necessary. The use of carbon

dioxide-free water in dissolving it is imperative, and its solution must be protected from the carbon dioxide of the air.

PREPARATION OF A GRADED SERIES OF BUFFERS FROM pH 2 TO 8

The titration graph of one of the acids listed above with sodium hydroxide using a glass electrode as indicator gave the clue for the preparation of buffers of any pH within its range. But it is not necessary for each investigator to make this titration graph over again. By using the special graph paper (Fig. 3) the titration graph becomes a straight line running through the pH value of the 50/50 buffer at its midpoint and a point 2 pH units to the left at the lower margin. The unit denoted by α on the left margin denotes the fraction of the acid neutralized by sodium hydroxide. Because this titration graph assumes that the free acid is undissociated, it cannot be used for any but very low concentrations of hydrogen ions, certainly not for pH less than 2.

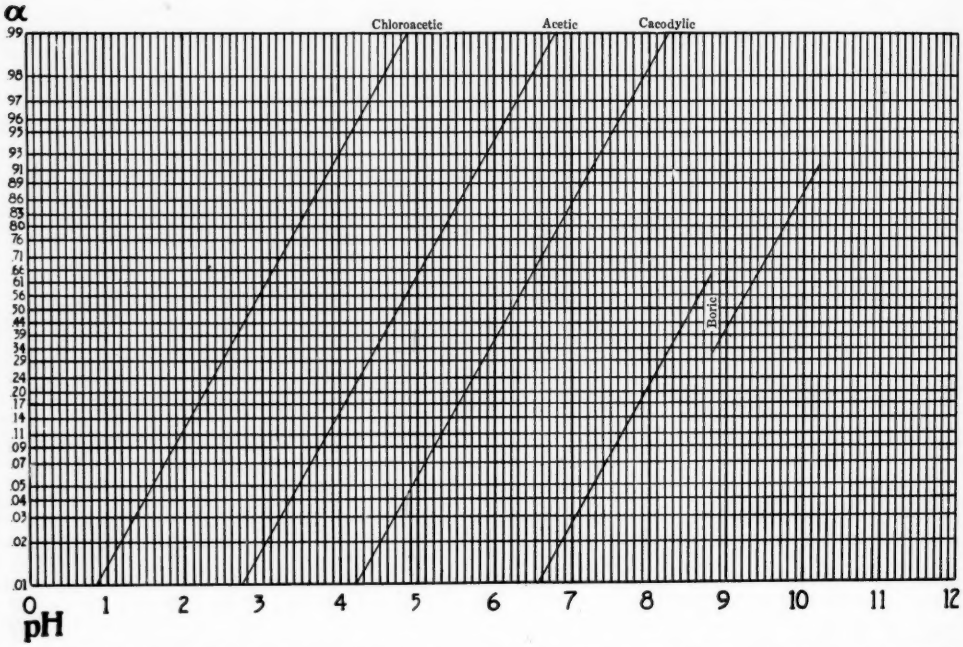

Fig. 3.—Titration graphs at 25° C. of chloracetic, acetic, cacodylic, and boric acids. The fraction neutralized is represented by α on the left. Hitchcock and Taylor[3] give slightly different values. The boric curve on the left assumes borax as completely, and on the right, as half neutralized.

From Fig. 3 it is seen that cacodylic acid covers the pH range from 4.2 to 8.2. In order to make a buffer of any pH within this range without calculations it is necessary for the "normality" of the normal sodium hydroxide and the cacodylic acid to be exactly equal. Therefore, pipette 10 c.c. of cacodylic acid into a titrating vessel, add one drop of phenolphthalein solution, and titrate with the sodium hydroxide to the maximum color that remains twenty seconds after stirring. Read the burette, and if 11 c.c. (for instance) have been required, add 10 per cent distilled water to the remainder of the cacodylic acid; if 9 c.c. (for instance) have been required, add 1 c.c. of water to each 9 c.c. of sodium hydroxide solution.

To prepare any buffer between pH 4.2 and 8.2 trace the pH coordinate to the cacodylic acid graph and note the a coordinate that it intercepts. If x represents the number of cubic centimeters of cacodylic acid chosen, add ax cubic centimeters of sodium hydroxide solution. Similar procedures may be made with acetic and chloracetic acids.

Boric acid behaves in a peculiar manner (Fig. 3). If we assume boric acid to be monobasic and titrate normal boric acid with normal sodium hydroxide, a straight line from about pH 8.8 to 10.2 is obtained, with pH 9.15 representing half neutralization (as borax). But if we assume borax to be the fully neutralized "salt," then boric acid is only half monobasic, and when it is titrated with 0.5 normal sodium hydroxide, the straight line from about pH 6.5 to 8.8 is obtained. This abnormal behavior, together with the sensitivity of borate buffers to ionic strength and the rapidity with which they absorb carbon dioxide from the air, makes the use of borates undesirable.

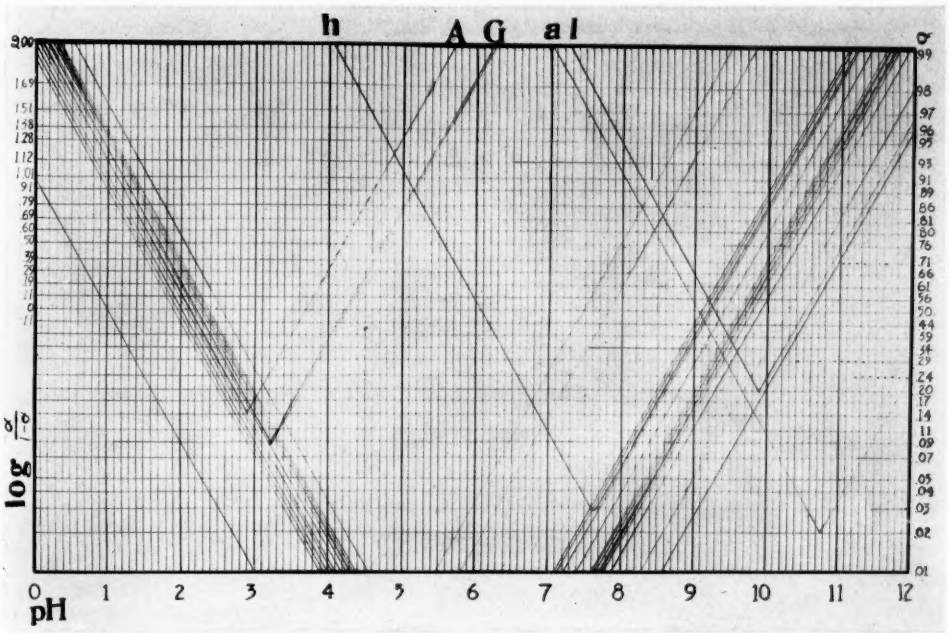

Fig. 4.—Ionization graphs at 25° C. of the amino acids of protein hydrolysates. *A*, aspartic acid; *h*, histidine; *G* (double line), glutamic and hydroxyglutamic acids; *a*, arginine; and *l*, lysine. The ionization graphs of the amino groups slope in the opposite direction to those of the carboxyl groups.

BUFFER ACTION OF AMINO ACIDS

In attempting to buffer solutions containing protein hydrolytic products one should bear in mind that they themselves have a powerful buffer action, as shown in Fig. 4. In this chart aspartic acid is marked *A;* glutamic and hydroxyglutamic, *G;* histidine, *h;* arginine, *a;* and lysine, *l*. The dissociation graphs of the other amino acids are so close together as to make labeling difficult. On the addition of any buffer composed of partly neutralized acid, the acid would partly combine with the amino groups of these amino acids and the base would distribute itself between the carboxyl groups of these amino acids and the

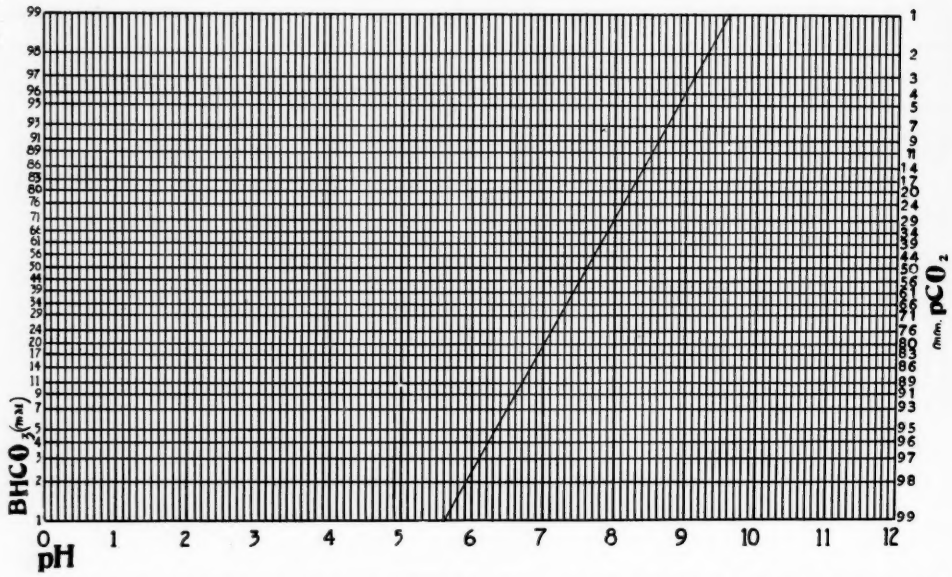

Fig. 5.—Carbonic acid-bicarbonate graph at 38° C. On the left is marked the bicarbonate; on the right, the partial pressure of carbon dioxide necessary to produce the pH given at the bottom.

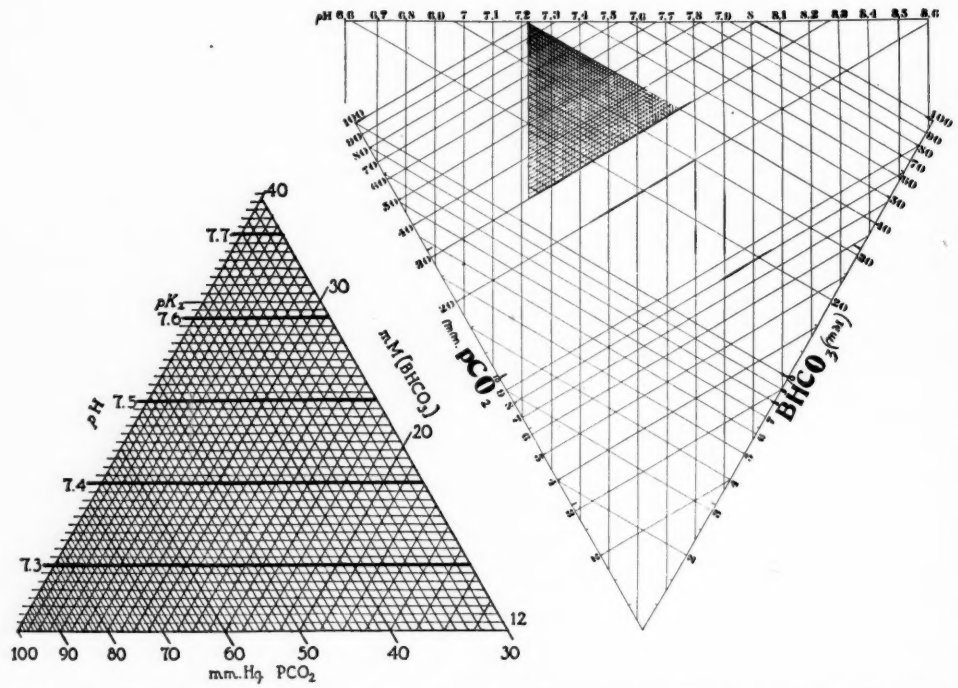

Fig. 6.—Graph of relation at 38° C. between the concentration of bicarbonate in millimols per liter, the pressure of carbon dioxide in millimeters of mercury, and the pH. The smaller triangle on the right is enlarged on the left to emphasize the physiologic range. The graph on the right starts at 1 mm. carbon dioxide pressure; the atmospheric pressure is about 0.2 mm. Whenever Ringer's fluid, for instance, containing bicarbonate is in contact with living tissue, the carbon dioxide pressure tends to be higher than atmospheric, and perhaps the range is sufficient for ordinary laboratory conditions.

added buffer acid. It is much simpler to use the buffering power of the amino acids themselves, adjusting the pH by additions of sodium hydroxide (or hydrochloric acid) with the aid of an indicator or glass electrode. In this chart the amphoteric ions (zwitterions) are represented as undissociated molecules since they do not affect the pH although they do affect the ionic strength.

BICARBONATE BUFFERS

Just as borate buffers may change by absorption of carbon dioxide from the air, bicarbonate buffers may change by loss of carbon dioxide to the air and can be preserved only if they are kept under a fixed partial pressure of carbon dioxide. Fig. 5 is a graph at 38° C. of pH of bicarbonate solutions of concentration shown on the left margin; pressure of carbon dioxide is given on the right margin. This graph is limited, however, by the fact that there is only one pressure of carbon dioxide for each concentration of bicarbonate. To extend the range the triangular form of graph shown in Fig. 6, right, may be used with the physiologic range enlarged at the left.*

REFERENCES

1. Armstrong, W. D.: Microdetermination of Fluorine, Indust. & Engin. Chem. **8:** 384, 1936.
2. Hulett, G. A., and Bonner, W. D.: A Method for the Preparation of Hydrochloric Acid Solutions, J. Am. Chem. Soc. **31:** 390, 1909.
3. Hitchcock, D. I., and Taylor, A. C.: A Standardization of the pH Scale at 38°, J. Am. Chem. Soc. **60:** 2710, 1938.
4. Kober, P. A., and Marshall, T. J.: Phenolphthalein and Its Colorless Salts, J. Am. Chem. Soc. **33:** 59, 1911.
5. Kolthoff, I. M.: The "Salt Error" of Indicators in the Colorimetric Determination of pH, J. Physiol. Chem. **32:** 1820, 1928.
6. McClendon, J. F.: A Direct Reading Potentiometer for Measuring Hydrogen-Ion Concentrations, Am. J. Physiol. **38:** 186, 1915.
7. —: Chart for the Conversion of Colorimetric Readings Into Hydrogen-Ion Concentration, J. Biol. Chem. **54:** 647, 1922.
8. —: The Determination of Hydrogen Ions in the Gastric Contents, J. Biol. Chem. **59:** 437, 1924.
9. —: Hydrogen Ion Determination, Am. Naturalist **64:** 289, 1930.
10. —: On the Graphic Representation of Ionic Equilibria in Blood Serum, Science **81:** 569, 1935.
11. —, and Medes, G.: Physical Chemistry in Biology and Medicine, Philadelphia, 1925, W. B. Saunders Co., pp. 110-112.
12. —, Russell, S., and Tracy, E.: The Determination of Hydrogen Ions in the Blood With the Aid of the Duboscq Colorimeter and Orthochrome-T or Paranitrophenol, J. Biol. Chem. **70:** 705, 1926.
13. Michaelis, L., and Gyemant, A.: Die Bestimmung der Wasserstoffzahl durch Indicatoren, Biochem. Ztschr. **109:** 165, 1920.
14. Rosenstein, L.: Ionization Constant of Phenolphthalein, J. Am. Chem. Soc. **34:** 1117, 1912.

*McClendon (1935). This triangular graph paper may be purchased at Hahnemann Medical College, 235 N. 15th Street, Philadelphia.

THE MEASUREMENT OF THE CHLOROFORM-SOLUBLE FRACTION OF BILIRUBIN IN PERSONS WITH JAUNDICE AND ITS SIGNIFICANCE*

NORMAN HEILBRUN, M.D., AND ROGER S. HUBBARD, PH.D.
BUFFALO, N. Y.

IT IS well known that the bilirubin in serum or plasma of patients with obstructive or hepatogenous jaundice gives a different reaction with diazotizing (van den Bergh) reagents from that given by bilirubin in specimens from persons with acholuric jaundice.[1] This can be demonstrated by noting the speed with which a color appears after the reagents and serum are mixed. It has been postulated that the difference in the reaction is due to two different types of bilirubin, but the underlying chemical or physicochemical causes of the difference have not as yet been definitely determined. That both types of pigment are present in some cases of jaundice has been suggested by some investigators.[2] In a recent paper we showed that an interpretation of the so-called biphasic van den Bergh reaction obtained when both pigments are present is often very difficult.[3] It seemed desirable, therefore, to investigate another method of distinguishing between the two forms of bilirubin.

Many years ago Grunenberg[4] observed that the bilirubin was wholly soluble in chloroform in persons who gave an indirect, slow van den Bergh reaction. Since this report attempts at the separation of two types of bilirubin in the serum by means of chloroform have been made by Varela and his coworkers,[5, 6] and more recently by Greene and associates.[7] The former workers studied various types of jaundice, especially the hepatogenous and the obstructive groups, and commented upon the different amounts of the chloroform-soluble fraction. In their analyses the chloroform-soluble portion was concentrated by evaporation after extraction, redissolved in alcohol, and diazotized. The residue which contained the water-soluble or direct-reacting portion was also diazotized, and the sum of the two fractions then gave the total amount of bilirubin in the serum.

In trying to repeat this work according to their technique we encountered certain difficulties. The diazotization of the two fractions did not always yield colors that could be easily compared with that of the cobalt standard. This same difficulty has been commented upon by many workers who have used the van den Bergh reaction, and various modifications of the test for avoiding the difficulty have been proposed. We tried several of these but found that they were not completely satisfactory. Our preliminary experiments showed that slightly acidified solutions of pure bilirubin in chloroform could be accurately quantitated by comparison with the 1:6,000 water solution of potassium di-

*From the School of Medicine, University of Buffalo and the Buffalo General Hospital.
Received for publication, December 27, 1939.

chromate standard used by Ernst and Förster.[8] Since this method did not involve diazotization, the difficulty mentioned was avoided.

When the yellow chloroform-soluble pigment was concentrated according to the technique of Varela and his co-workers, and redissolved in chloroform and alcohol to a definite volume, it was found on comparison with the dichromate standard, both before and after concentration, that there was a loss of approximately 50 per cent of the yellow color. Because of this it was decided not to concentrate the extract but to use a direct comparison between the yellow color given by the chloroform-soluble pigment and the dichromate standard. The accuracy of this determination was checked in two ways: first, by measurement of the color of known amounts of bilirubin dissolved in chloroform as previously mentioned; second, by demonstrating the quantitative recovery of injected bilirubin. For this bilirubin was injected into normal persons or those with hypochromic anemia. Samples of blood were taken both before and within three to five minutes after the injection. The quantity of bilirubin found in the chloroform extract of the sample after injection compared closely with the total increase of the bilirubinemia as estimated by the quantitative van den Bergh test. When such samples were tested with the qualitative van den Bergh reaction, it was found that the reaction was always of the indirect or delayed type. The results of these experiments have been described in a previous paper.[3]

The study of a group of patients with increased bilirubinemia due to various causes selected from the services of the Buffalo General Hospital is presented here. All specimens of sera or plasma were subjected to chloroform extraction, and the total amount of the chloroform-soluble bilirubin fraction was estimated. The bilirubin in a second portion of the serum or plasma was determined quantitatively by the Thannhauser and Andersen method.[9] The relationship between the quantity of the chloroform-soluble or indirect-reacting bilirubin and the total amount were charted in an attempt to see whether there were any correlations between the various types of jaundice, and whether the obstructive or hepatogenous groups could be separated from one another. The latter, from a clinical point of view, would be very desirable.

The technique for the estimation of the chloroform-soluble fraction, which can be employed even in the presence of a moderate degree of hemolysis, is as follows: 1 c.c. of the serum or plasma is placed in a centrifuge tube. Three to 4 c.c. of chemically pure chloroform is then added. The tube is capped with the thumb and shaken vigorously for twenty to thirty seconds. It is then centrifuged for two to three minutes. The lower yellow chloroform layer is transferred to a graduated 15 c.c. centrifuge tube. This is done with a fine-tipped pipette inserted through the upper thick layer down to the bottom of the tube. Extraction is repeated until the chloroform remains colorless. Each successive fraction is added to the original in the graduated tube. Usually two or three extractions will remove all of the soluble pigment; occasionally, five or six are necessary. The total amount of chloroform-soluble bilirubin is now estimated in the following manner: The total quantity of the pooled fractions in the graduated tube is noted. Turbidity is rarely present, but when it is it can be cleared by placing the tube in a warm water bath for a few seconds. A small portion of this is now removed, and its color is compared

with that of the 1:6,000 potassium dichromate solution in a microcolorimeter with cups of a capacity of 1 to 2 c.c. The following calculation was derived from our experiments, and is the same as that which Soffer[10] used in the bilirubin excretion test in which acetone is employed as the solvent:

$$\text{Total amount of } CHCL_3 \times \frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.329 = \text{Milligrams of bilirubin per 100 c.c.}$$

The patients studied have been arbitrarily divided into the following groups: (1) hepatogenous jaundice, which included so-called catarrhal jaundice, acute yellow atrophy, portal cirrhosis, toxic hepatitis due to arsphenamine, toxic hepatitis accompanying overwhelming bacterial sepsis; (2) obstructive jaundice due to common duct stone, carcinoma of the head of the pancreas, obstructive biliary cirrhosis; (3) mild jaundice accompanying chronic passive congestion; (4) hemolytic jaundice, including congenital acholuric jaundice, pernicious anemia and allied states, mild jaundice accompanying malarial therapy for syphilis of the central nervous system; (5) two patients who fall in the group described by Rozendaal, Comfort, and Snell[11] as functional jaundice which gives the indirect van den Bergh reaction. Some persons in most groups were studied by successive determinations through the course of the disease.

TABLE I

	GROUP	NUMBER OF DETERMINATIONS	AVERAGE VALUE OF TOTAL QUANTITY OF CHLOROFORM-SOLUBLE BILIRUBIN (MG./100 C.C.)	AVERAGE PERCENTAGE OF CHLOROFORM-SOLUBLE PIGMENTS TO THE TOTAL QUANTITATIVE VAN DEN BERGH TEST (PER CENT)
I	Hepatogenous	74	0.89	24.0
II	Obstructive	38	0.68	27.9
III	Chronic passive congestion	54	0.64	61.0
IV	Hemolytic	34	1.07	74.0
V	Functional	4	1.11	86.7

The average values of the chloroform-soluble portion and the average percentage of the total amount of bilirubinemia which it forms have been estimated for the various groups and recorded in Table I. In addition, the individual values for each specimen studied have been charted according to their frequencies. One chart records the total quantity of the chloroform-soluble fraction and the other records the percentage which it forms of the total amount of bilirubin as estimated by the quantitative van den Bergh reaction. Each dot represents a single determination.

COMMENT

It will be noted from Table I that there is little difference between the average values for the patients grouped under "hepatogenous" and "obstructive" jaundice. This is true both for the chloroform-soluble fraction and for the percentage which it forms of the total bilirubin. Any attempt to separate these two clinical conditions on the basis of these average values would not

seem to be justified. Furthermore, many persons in both groups with a high degree of bilirubinemia had relatively small amounts of chloroform-soluble bilirubin when compared with the total amount present. Nevertheless, even though this amount was a small proportion of the total, it was considerably greater than that found as a result of normal red blood cell destruction. If one considers the usual amount of bilirubin in the blood stream a result of the balance between normal red blood cell destruction and the ability of the liver to remove it, then the accumulation of it in the blood stream in the absence of increased red blood cell destruction should be some indication of the functional state of the liver. If this is true, then the measurement of the chloroform-soluble or indirect-reacting pigment in cases of severe jaundice may be regarded as an estimation of liver function. In essence, this would seem to be the same measure of function as that utilized in von Bergmann's[12] bilirubin excretion test, but this proposed chloroform method can be applied when jaundice is present.

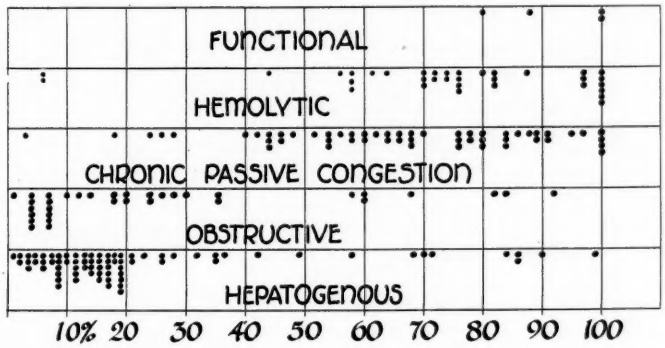

Chart. 1.—Percentage which the chloroform-soluble bilirubin forms of the total quantity of bilirubin as estimated by the quantitative van den Bergh reaction. Each dot represents a single determination.

Some persons had values of chloroform-soluble pigment, ranging from 1.5 to 3.5 mg. per 100 c.c., which amounted to only 15 to 30 per cent of the total amount of bilirubinemia. However, this quantity of pigment is three to six times the amount normally found, and, in our opinion, indicates an impairment of the ability of the liver to perform its normal excretory function. Furthermore, the frequency charts show that greater numbers of the higher values of the chloroform fraction occur in the hepatogenous group. It would seem reasonable that this should be so because it is in this group that the pathologic process involves the liver cells diffusely, and one should expect that the function of excreting the bilirubin derived from the breakdown of red blood cells would be impaired to a greater degree in this group than in obstructive jaundice where the primary process does not involve the liver cells directly. We feel that this confirms our belief.

That both fractions are present in persons with severe jaundice of this nature has been commented upon only recently.[13] That their presence was not recognized earlier when the qualitative van den Bergh test was done in the usual way resulted from the fact that the immediacy of the reaction is so marked in the presence of a relatively large amount of direct-reacting pigment, that the slowly progressive reaction of the indirect pigment is obscured.[3]

In the groups of persons with chronic passive congestion it is seen that the greatest portion of the total amount of bilirubin is chloroform soluble. In some cases the pigment was wholly of this type, and this was usually so when the congestion was at its height. Again it would seem reasonable to consider that the function of a markedly congested liver is impaired. It may be argued that some of the bilirubinemia resulted from the breakdown of infarcts. However, in two persons with rather high values subsequent autopsy showed no evidence of infarction, either recent or old. This increase of the chloroform-soluble pigment is also in accord with the fact that most of the cases give an indirect or biphasic van den Bergh reaction. We studied this latter reaction and found it to be the result of the combination of a relatively large amount of indirect-reacting pigment with a small amount of direct-reacting pigment.[3]

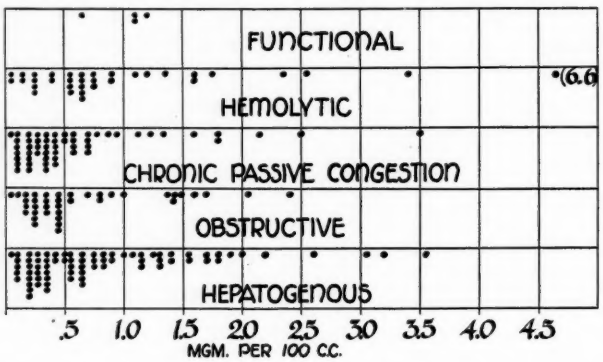

Chart 2.—Total quantity of chloroform-soluble bilirubin in each specimen. Each dot represents a single determination.

Those persons whose jaundice was due to excessive hemolysis were found to have the greater portion of the total amount of bilirubin in the chloroform-soluble form as seen in Table I and Charts 1 and 2. This coincides with the frequent observation that they give a delayed or indirect van den Bergh reaction. However, if the concentration of the total pigment is relatively high, a prompt color development may be noted upon addition of the diazotizing reagent, but this is always of the slowly progressive type, requiring fifteen to thirty minutes to reach its maximum intensity.

The cases in the last group of functional disability of the liver show the greatest portion of the pigment to be soluble in chloroform. This also conforms with the observed indirect van den Bergh reaction.

CONCLUSIONS

1. A convenient and simple method for determining quantitatively the solubility of bilirubin in chloroform has been described.

2. This method is applicable to the measuring of the so-called indirect or delayed-reacting pigment in persons with jaundice.

3. It is suggested that a measurement of this portion may serve as an index of the potential capacity of the liver when jaundice is present and when excessive hemolysis is absent.

REFERENCES

1. van den Bergh, A. A. Hijmans, and Muller, P.: Über eine direkte und eine undirekte Diazoreaktion auf Bilirubin, Biochem. Ztschr. **77**: 90, 1916.
2. Fiegl, J., and Querner, E.: Bilirubinamie in ihren physiologischchemischen Beziehungen mit besonderer Berucksichtigung der diagnostischen Bedeutung, Ztschr. f. d. ges. exper. Med. **9**: 153, 1919.
3. Heilbrun, N., and Hubbard, R. S.: An Experimental Study of the Biphasic van den Bergh Reaction, Am. J. Clin. Path. **8**: 273, 1938.
4. Gruneberg, K.: Ueber die Topik der Unwandlungsstatten der Chloroformloslichkeit des Bilirubins, Ztschr. f. d. ges. exper. Med. **35**: 128, 1923.
5. Varela, B., Recarte, P., and Esculies, J.: Méthode simplifiée pour la séparation et le dosage isolé des deux bilirubines, directe et indirecte, du sérum sanguin dans les ictères, Compt. rend. Soc. de biol. **108**: 1009, 1931.
6. Varela-Fuentes, B., and Viana, C.: Les bilirubines, directe et indirecte, du sérum dans les ictères chroniques. Valeurs données par leur dosage séparé, Compt. rend. Soc. de biol. **116**: 1187, 1934.
7. Greene, C. H., Plotz, M., and Localio, S. A.: Liver and Biliary Tract; Review for 1937, Arch. Int. Med. **61**: 655, 1938.
8. Ernst, Z., and Förster, K.: Über die Bestimmung des Blutbilirubins, Klin. Wchnschr. **3**: 2386, 1924.
9. Thannhauser, J. S., and Andersen, E.: Methodik der quantitativen Bilirubinbestimmung in menschlichen Serum, Deutsches Arch. f. klin. Med. **137**: 179, 1921.
10. Soffer, L. J.: Bilirubin Excretion as a Test for Liver Function During Normal Pregnancy, Bull. Johns Hopkins Hosp. **52**: 365, 1933.
11. Rozendaal, H. M., Comfort, M. W., and Snell, A. M.: Slight and Latent Jaundice; Significance of Elevated Concentrations of Bilirubin Giving Indirect van den Bergh Reaction, J. A. M. A. **104**: 374, 1935.
12. von Bergmann, G.: Zur Funktionellen Pathologie der Leber, insbesondere der Alkohol-Ätiologie der Cirrhose, Klin. Wchnschr. **6**: 776, 1927.
13. Snell, A. M., and Magath, T. B.: Use and Interpretation of Tests for Liver Function; clinical review, J. A. M. A. **110**: 167, 1938.

A SIMPLIFIED CONTINUOUS EXTRACTOR FOR ESTROGENS AND ANDROGENS*

DONALD WILSON, PH.D., ROBERT C. GRAUER, M.D., AND ELEANOR SAIER, B.S., PITTSBURGH, PA.

THOROUGHNESS in the extraction of urine being the first essential for the proper assay of a given specimen, we have developed a continuous extractor which embodies simplicity with economy without sacrificing efficiency. The extractor was designed on the basic principle of that employed by Koch[1] in his much larger extractor. It consists of an all-glass apparatus made up of five units; a round bottom flask with a $\frac{40}{35}$ joint which can be made interchangeable with flasks of various sizes, an Erlenmeyer liter flask of heavy glass suitable for vacuum work, an ordinary Hopkins condenser, a side arm and upright portion connecting the first three mentioned units, and a filter tube containing a sintered glass bottom. The various measurements of the apparatus† are given in Fig. 1. The extractor can be used singly or in a battery of as many units as is desired. We found that a battery of four extractors makes a

*From the Endocrine Laboratory of the Elizabeth Steele Magee Hospital and the Department of Research in Endocrinology and Metabolism of the William H. Singer Memorial Research Laboratory of the Allegheny General Hospital, Pittsburgh.

Received for publication, December 11, 1939.

†This unit can be secured from the Scientific Glass Co., Bloomfield, N. J.

practical set-up and that if necessary multiple batteries of four each can be placed in position, each battery facing another. To facilitate working with a battery, an extractor rack was designed from ⅜ inch gas pipe to which a vacuum line, water line, and overflow line for the water were permanently attached. Each unit was connected separately to its own three lines, and the vaccum was connected with a common vacuum pump. An adjustable bar which can be raised or lowered holds the clamp that fastens the round bottom flask securely. A battery of four extractors occupies a space 26 inches in width and only 22 inches in depth, including the space occupied by the electric hot plate,* which is long enough for four flasks. Figs. 2 and 3 give the details of design and show the compactness of the battery.

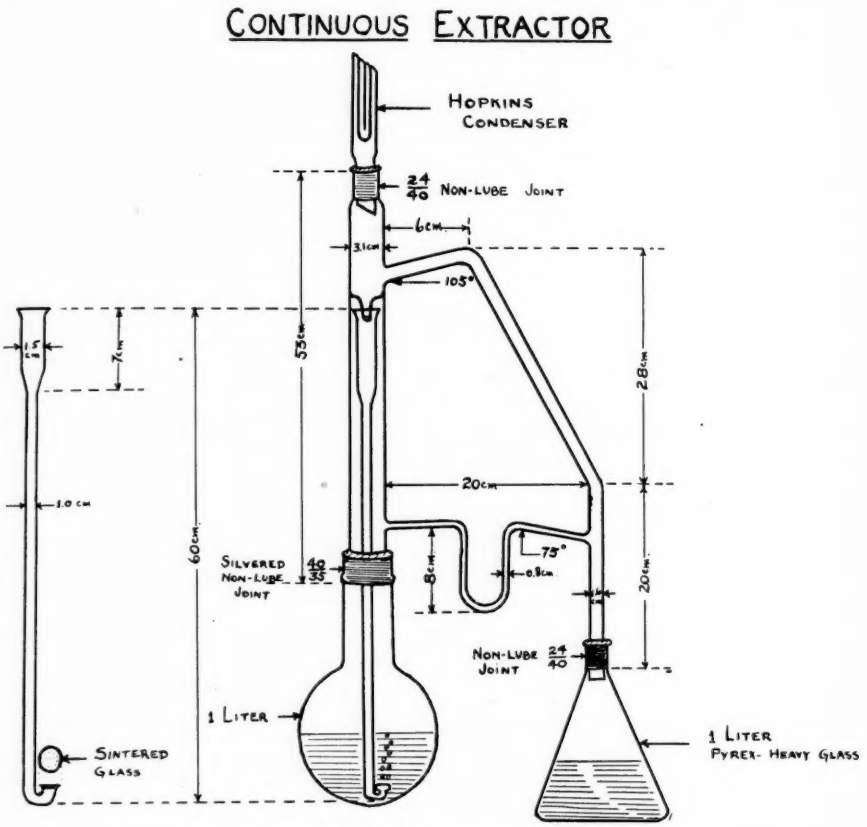

Fig. 1.—Details of construction extractor.

The methods employed in our laboratories are modifications of generally accepted methods.[2, 3] The apparatus can be used for the extraction of both estrogens and androgens. Seven hundred and fifty cubic centimeters of previously hydrolyzed urine are placed in the round bottom flask, and a like amount of benzene is introduced into the Erlenmeyer flask which is placed on the hot plate. By using a vacuum we were able to speed up the volatilization of the benzene and thus hasten the extraction. The side arms and the inner filter tube are arranged so that all of the volatilized and condensed benzene must pass through the urine that is being extracted. The incline on the distal arm of the

trap aids siphoning of the condensed benzene back into the Erlenmeyer flask and a continuous process proceeds. The sintered glass on the filter tube acts as a diffusing medium so that a maximum amount of contact between the benzene and urine ensues.

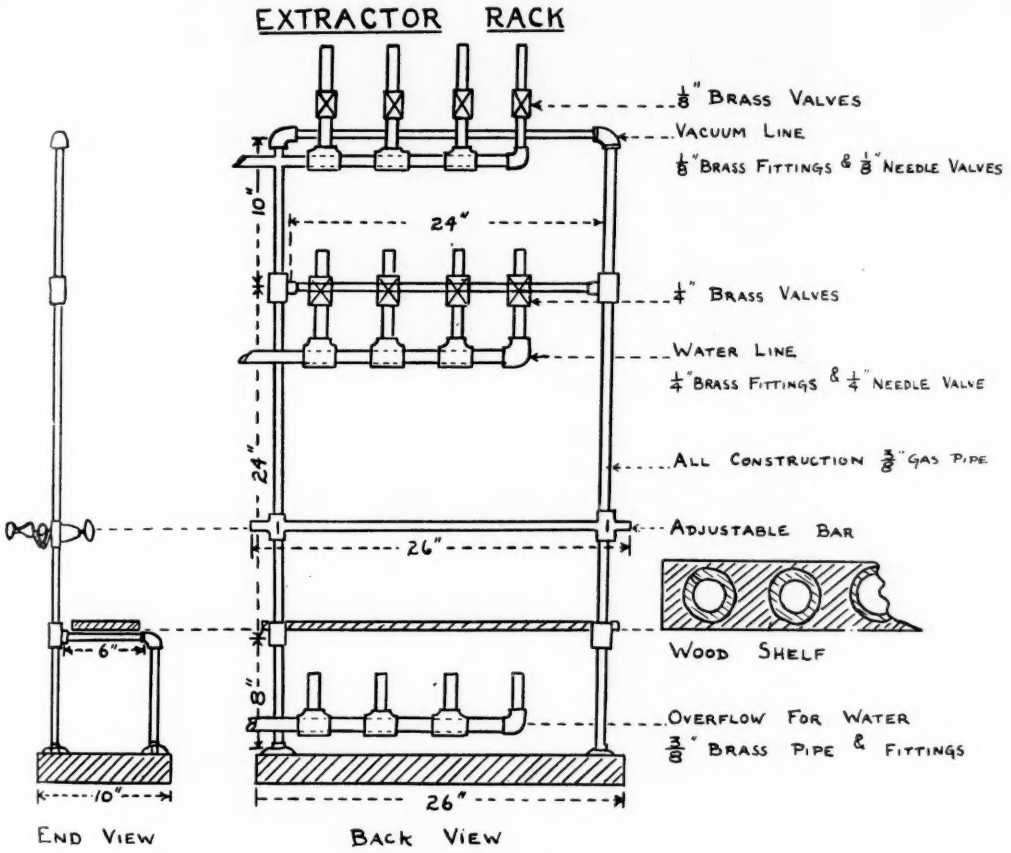

Fig. 2.—Details of construction of extractor rack.

It has been suggested[4] that approximately twelve volumes of benzene should be passed through the urine to be extracted in order to secure the maximum yield. It has further been observed that for androgenic extraction a shortening of the period of extraction is productive of a higher yield.[5, 6] In the case of extraction for estrogens this does not appear to be a factor, the preliminary length of hydrolysis appearing to be the determining point in the yield.[7] Nevertheless, whether estrogens or androgens are being extracted, the ability to complete an extraction during the working hours is important. Where inflammable or explosive materials are being used, the fire hazard is too great to allow an extraction to proceed throughout the night. We computed that seven liters of benzene can be passed through the urine in eight hours so that ten volumes of benzene, considering volume for volume, can be passed through the hydrolyzed urine. While the reduction of the time is not a factor in the quantitative yield of estrogen, it is definitely an important factor in producing a higher androgenic yield.

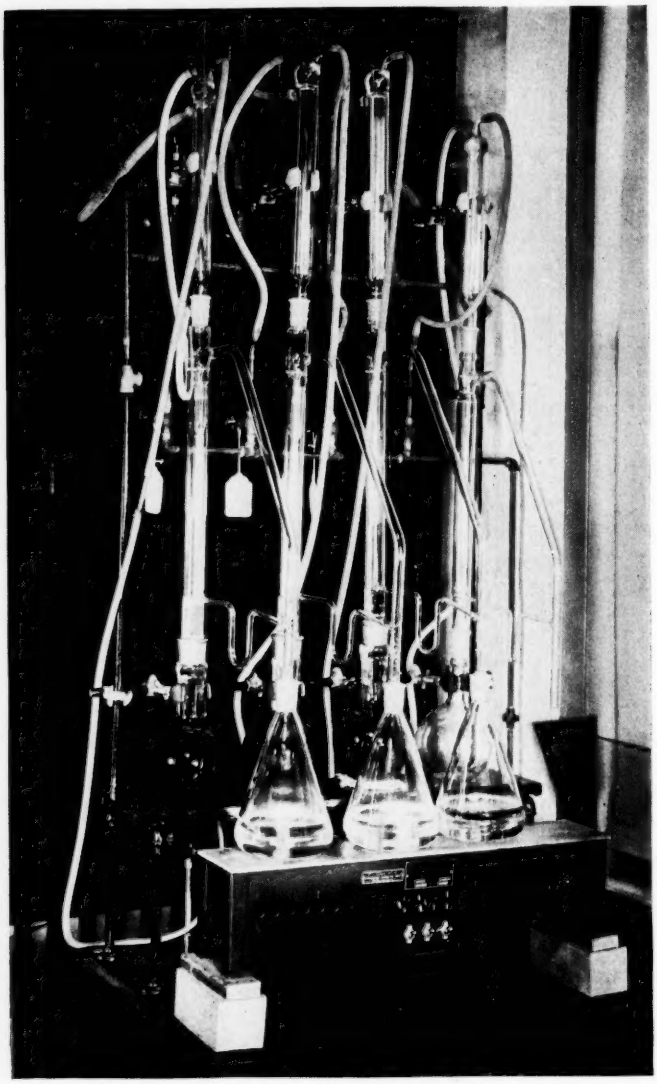

Fig. 3.—Photograph of extractors and extractor rack arranged as a battery of four extractors.

CONCLUSIONS

1. A simplified, economical, and efficient continuous extractor for estrogens and androgens is described.

2. An extractor rack to permit economy of space in arranging batteries of extractors is illustrated.

3. The principle of safety and efficiency in extraction is emphasized.

REFERENCES

1. Gallagher, T. F., Koch, F. C., and Dorfman, R. I.: Procedure for Quantitative Extraction of Sex Hormones From Urine, Proc. Soc. Exper. Biol. & Med. **33**: 440, 1935.
2. Gallagher, T. F., Peterson, D. H., Dorfman, R. I., and Koch, F. C.: The Daily Urinary Excretion of Estrogenic and Androgenic Substances by Normal Men and Women, J. Clin. Investigation **16**: 695, 1937.

3. Wilson, D., and Strickler, H.: Unpublished data.
4. Gallagher, T. F., Peterson, D. H., Dorfman, R. I., and Koch, F. C.: The Daily Urinary Excretion of Estrogenic and Androgenic Substances by Normal Men and Women, J. Clin. Investigation **16**: 695, 1937.
5. Neudstadt, Rudolph: Photo-Colorimetric Method for the Determination of Androsterones in Urine, Endocrinology **23**: 711, 1938.
6. McCullagh, D. Roy, and McLin, T. Roland: The Extraction of Androgens From Urine, Endocrinology **23**: 711, 1938.
7. Grauer, R. C., and Saier, Eleanor: Unpublished data.

THE DETERMINATION OF CALCIUM IN URINE*

A. E. Sobel, B.S., Ch.E., M.A., Ph.D., and B. A. Sobel, B.Ch.E.
Brooklyn, N. Y.

THE estimation of calcium in the urine is usually more difficult than the estimation of serum calcium. This is due to the tendency of urates and other organic materials to coprecipitate with the calcium oxalate precipitate and thus interfere in the subsequent oxidimetric titration. By employing the direct acidimetric titration principle,[1, 2] it was found that excellent results may be obtained in urine without resorting to ashing. In this procedure the calcium oxalate is converted to the carbonate or to the oxide by heat, which causes the decomposition of any adsorbed urates or other organic materials. To prove this point, comparison analyses were performed on urine, both directly and upon hydrochloric acid solution of the residue obtained by dry ashing. To prevent coprecipitation of magnesium and eliminate any possibility of phosphate precipitation, the pH of the solutions was adjusted to from 3.0 to 3.3 for precipitation of the calcium oxalate. The results are shown in Table I. Each value is the mean of two or more analyses on the same solution.

Table I

Comparison of Calcium Values Obtained on Ashed and Unashed Urine
(values expressed in milligrams of calcium per 100 ml. of urine)

UNASHED	ASHED
9.15	9.15
11.70	11.75
4.14	4.12
12.0	12.05
4.02	3.98
4.26	4.32

METHOD

For urine specimens without sediment. Two to 4 ml. of urine are pipetted into a 15 ml. pyrex ungraduated centrifuge tube. One milliliter of saturated ammonium oxalate is added. A drop of 0.04 per cent thymol blue indicator is added, and the pH is adjusted to from 3.0 to 3.3 by the use of 6 normal

*From the Pediatric Research Laboratory and the Division of Biochemistry, the Jewish Hospital of Brooklyn.
Received for publication, February 1, 1940.

hydrochloric acid and ammonium hydroxide. The solution is allowed to stand for three hours. From this point on the method follows exactly that of Sobel and Sklersky for blood serum.[1] The oxalate is converted to carbonate by heating at 475° to 525° C. The carbonate is then dissolved in a hot 10 per cent boric acid solution and directly titrated with 0.01 N hydrochloric acid.

For urine specimens with sediment. In some urines, and in all urines after standing, a precipitate appears. This can be cleared up by boiling a representative sample of the urine plus sediment, or the entire specimen if the sediment is coarse-grained, preventing a representative sample from being taken, with concentrated nitric acid in the proportions of 10 ml. of acid to 50 ml. of urine. After boiling to one-half the original volume, the digest is diluted to known volume and a sample is analyzed as above.

Preparation of ashed urine specimens. To compare the calcium values obtained on ashed urine specimens to that of unashed urine, the following technique was employed: twenty-five milliliters of urine are pipetted into a platinum dish. The liquid is then evaporated off on a steam bath or in an oven at 100° C. overnight. The residue is then ashed in an oven at 500° to 600° C. until white. The ash is dissolved in 6 N hydrochloric acid and transferred quantitatively to a 25 ml. volumetric flask. Two milliliters of this solution are then used for analysis.

CONCLUSION

The direct acidimetric titration method for calcium is applicable to urine without ashing. This simplifies the methods for determining calcium in urine.

REFERENCES

1. Sobel, A. E., and Sklersky, S.: Direct Acidimetric Microtitration Method for Calcium, J. Biol. Chem. **122**: 665, 1938.
2. Sobel, A. E., and Sobel, B. A.: Microestimation of Calcium in Serum, J. Biol. Chem. **129**: 721, 1939.

THE SHORT PHENOLSULPHONEPHTHALEIN TEST OF RENAL FUNCTION*

MILTON PLOTZ, M.D., F.A.C.P., AND MARGARETHE ROTHENBERGER, M.D.
BROOKLYN, N. Y.

WITH THE TECHNICAL ASSISTANCE OF EDGAR FERGUSON, B.S., AND VICTOR GINSBURG, M.D.

IT MAY be safely stated that at the present time there is no altogether satisfactory test of kidney function. There is reason to believe that moderate or even advanced renal lesions may produce no modification in kidney function tests and that a kidney which is normal in every way may on occasion give a deceivingly low reading. Evaluation of the patient's renal status means a consideration of his history, physical examination, examination of urine, blood, ocular fundi, and other routine examinations as well as the application of certain renal function tests. All the data must be considered and all the evidence weighed before any final judgment can be made and, even then, it must usually be made with caution unless the disease process is advanced. Don's conclusions, after using several different methods of testing renal function on 63 patients, is worth quoting.[1] "The lack of parallelism between the tests especially in the lesser grades of renal damage, was most disappointing. None of them appear to be capable of enabling the clinician to give a very much more accurate prognosis than is possible from the knowledge of the blood urea alone. However, if any of the tests show marked loss of renal function in the absence of a raised blood urea it would probably be best to give a guarded prognosis. A re-examination of the patient in three months might help; clinical examination will often furnish very valuable information and must still be regarded of the foremost importance. To conclude, the failure of the tests to agree closely with one another suggests either that they are not completely reliable or that different renal functions are being measured."

In view of the fact, therefore, that no one test of kidney function can provide a short cut to diagnosis, it seemed desirable to find one of the numerous tests now available which could be applied with a minimum of technical equipment and experience, with as little consumption of time as possible and would yet correlate fairly closely with more cumbersome methods. The criteria for a test of this sort seemed to be met by the fractional phenolsulphonephthalein test popularized by Chapman,[2] which can be completed in half an hour, requires a minimum of apparatus and reagents and can be used as a simple office procedure if necessary. Genitourinary surgeons have until very recently utilized the phenolsulphonephthalein test to a far greater extent than have internists, and it was they who first called attention to the importance of the

*From Kings County Hospital, Long Island College of Medicine Division, the medical services of Dr. Tasker Howard and Dr. Carl H. Greene.

This study was made possible by a generous grant from the Harriet Weinbaum Foundation.

Clinical studies in renal disease No. 5 from the Long Island College of Medicine.

Received for publication, February 3, 1940.

early excretion of the dye.[3] More recently, MacKay and Rytand have written: "The phenolsulphonephthalein test is superior to the blood urea concentration as a measure of the amount of functioning renal tissue."[5]

In an effort to repeat and confirm Chapman's experiments and to establish a series of satisfactory normals, the following project was carried out on the medical wards of the Kings County Hospital. Two hundred and three cases were studied in detail, of which 109 had obvious kidney damage. The fractional phenolsulphonephthalein test was correlated with the two-hour phenolsulphonephthalein, urea clearance, concentration tests, studies of nitrogen retention in the blood, and clinical data, although not all tests were done in every case. A detailed study of the sodium ferrocyanide test, as proposed by Stieglitz, was done simultaneously and has already been reported in detail in a separate paper.[4]

The technique of the test suggested by Chapman was followed by us with occasional modifications to be noted. The patient voids in the morning and drinks two glasses of water, noting the time. This may be done before reporting, but it is best to perform the entire test in the laboratory or office. One-half hour later 1.0 c.c. (6.0 mg.) of phenolsulphonephthalein solution is injected intravenously, and exactly fifteen and thirty minutes later the patient voids and the urine is collected. For the purposes of this experiment, one- and two-hour specimens were also collected, but this is unnecessary for routine examinations since no additional information is obtained by prolonging the test period. For use in the physician's office, when saving time is important, the total test period can be shortened from one hour to one-half hour in the following manner: When the patient arrives, give him two or three glasses of water to drink at the same time the injection is made and collect only the half-hour specimen if the patient cannot urinate at the end of fifteen minutes. The results are somewhat improved if the patient is told to drink a glass of water before leaving home. He should not void before the dye is given. We have found no significant differences between the two methods.

Vallery-Radot and his colleagues[6] were favorably impressed by the short test and have abandoned longer procedures. In their tests all normal patients excreted 25 per cent or more within fifteen minutes. Readings of 15 per cent or less indicated some degree of failure of kidney function in almost all cases. They considered readings of about 20 per cent to be equivocal and repeated the tests. Part of the purpose of our experiment was to check these figures and to establish satisfactory normals for phenolsulphonephthalein excretion, and while we are not as yet wholly satisfied about this aspect of our work, we feel that our criteria are sufficiently accurate for most clinical purposes.

Normals.—One hundred and twenty-three short phenolsulphonephthalein tests were performed on 103 adult patients thought to be clinically free of renal disease or hypertension. In fourteen instances the phenolsulphonephthalein excretion after fifteen minutes was less than 25 per cent. In eight of these, one or more confirmatory tests (urea clearance, blood urea, ferrocyanide excretion) supported the conclusion that there was, temporarily at least, some impairment of renal function. It is possible, of course, that some of these patients will go on to clinical renal disease and that in any group of hospital patients presumably free of kidney involvement a small percentage would show some

changes in renal function tests. Of the remaining six patients, one showed a normal reading on repetition of the test. In five cases (4.9 per cent), in which there was no supporting evidence of renal impairment, the phenolsulphonephthalein readings were subnormal. The test gave a slightly higher percentage of normal figures in normal patients than any of the other tests, urea clearance, concentration tests, ferrocyanide clearance, blood urea, and two-hour phenolsulphonephthalein. In no case where the figure was low did the two-hour phenolsulphonephthalein test reveal normal figures. In two cases the figures were below normal for the quarter-hour excretion but were normal for the thirty-minute period.

Kidney Disease.—There were 21 cases of definite kidney impairment. The diagnoses in all cases were either chronic glomerulonephritis or nephrosclerosis. No tests were performed on patients with acute kidney disease. The fifteen-minute phenolsulphonephthalein test was 20 or below in nineteen of these. In one of the two others the figure was 22 per cent and in the other it was 25 per cent. In the former case 74 per cent of the dye was excreted in two hours. In the latter, the fifteen-minute figure was apparently normal but very little more dye was excreted in two hours, the total for that period being 35 per cent. The urea clearance was constantly diminished in all cases.

Hypertension.—There were 26 persons with early to moderate hypertension without clinical evidence of cardiac or renal failure. In eight of these the fifteen-minute phenolsulphonephthalein test showed diminished excretion. In only five was the urea clearance reduced below normal limits. This series is, of course, too small to be of much value, but it would seem to indicate that this test is a fairly sensitive method of detecting early renal changes in hypertension.

Congestive Heart Failure.—There were 20 patients with congestive heart failure. In all but two of these, during the stage of congestion there were low figures which improved as the cardiac function returned to normal. The urea clearance and the ferrocyanide excretion were correspondingly diminished. The blood nitrogen levels were not as consistently affected. Urine concentration tests were of little value during congestive heart failure.

There are few precautions needed in making or interpreting this kidney function test. We observed no reactions to the intravenous use of this dye, although Vallery-Radot, while temporarily using a somewhat different technique, did observe some. In the presence of liver damage the excretion is somewhat in excess of what one would expect, and borderline results should be interpreted conservatively and repeated. The explanation of this phenomenon probably lies in the fact that the liver usually excretes a portion of the phthaleins in the body through the biliary tract, and when there is failure of this mechanism, higher amounts appear in the urine. We have no experience with the test in uncomplicated anemia, but Chapman maintains that there is no significant effect of the latter on phenolsulphonephthalein excretion.[7]

Catheterization was rarely necessary. If the patient did not void after fifteen minutes, an extra glass of water was given and only the thirty-minute specimen was collected with little or no impairment of the test's value. Catheterization is, of course, an objection to any test which is to be used as an office procedure, but it may be necessary in prostatic enlargement or other conditions

in which there may be residual urine. The phenolsulphonephthalein test shares this disability naturally with any test which depends on the measurement of urinary constituents and is, therefore, necessarily limited in usefulness in cases of urinary retention. It should be noted that Macht[8] maintains that phenolsulphonephthalein elimination may be delayed after the ingestion of magnesium sulfate for a period of as long as three to four hours. We have no experience with this.

Stieglitz[9] cautions that "In rare instances some metabolic quirk causes chemical destruction of the dye within the body. If the urine contains no dye after injection, this is then no proof of renal failure." We have had no such experience but the caution should be borne in mind.

CONCLUSIONS

The short phenolsulphonephthalein test has been a very valuable aid to clinical diagnosis in our hands. The two-hour period has no advantage over the shorter periods. The positive value of the test is greater than its negative value. In other words, one is safe in saying that a patient who excretes 25 per cent or more phenolsulphonephthalein within fifteen minutes after its administration, or more than 35 per cent within thirty minutes, has normal kidney function. On the other hand, failure to secrete this amount has less diagnostic value. Borderline cases (17 to 24 per cent in fifteen minutes or 25 to 34 per cent in thirty minutes) should be evaluated with caution or the test should be repeated. Although definitely low reading almost surely means impaired kidney function at the time of the test, low readings should not be allowed to supersede clinical judgment.

SUMMARY

1. The difficulties of measuring renal damage by means of kidney function tests are described.

2. The short phenolsulphonephthalein (P.S.P.) test takes a half hour or less and is extremely useful. It is simple, requires a minimum of technical skill and equipment, and may be used as an office procedure. The two-hour phenolsulphonephthalein test has no advantages over the shorter test.

REFERENCES

1. Don, C. S. D.: Tests for Renal Function, Brit. M. J. **2**: 54, 1937.
2. Chapman, E. M., and Halsted, J. A.: The Fractional Phenolsulphonphthalein Test in Bright's Disease, Am. J. M. Sc. **186**: 223, 1933.
   Chapman, E. M.: Further Experience With the Fractional 'Phthalein Test, New England J. Med. **214**: 16, 1936.
3. Shaw, E. Clay: A Study of the Curve of Elimination of Phenolsulphonphthalein by the Normal and Diseased Kidneys, J. Urol. **13**: 575, 1925.
4. Plotz, M., and Rothenberger, M.: Sodium Ferrocyanide Excretion in the Estimation of Renal Function, J. Lab. & Clin. Med. **24**: 844, 1939.
5. MacKay, E. M., and Rytand, D. A.: Significance of the Phenolsulphonphthalein Test of Renal Function, Arch. Int. Med. **55**: 131, 1935.
6. Vallery-Radot, P., Delafontaine, P., Israël, R., and Porge, J.: L'epreuve de la phénolsulfonephtaléine en un quart d'heure, Presse méd. **44**: 2009, 1936.
7. Chapman, E. M.: The 'Phthalein Test of Kidney Function in Anemia, New England J. Med. **213**: 166, 1935.
8. Macht, D. I.: Concerning the Effect of Saline Purgatives on the Absorption and Excretion of Phenolsulphonphthalein, J. Urol. **7**: 271, 1922.
9. Stieglitz, E. J.: Clinical Application of Renal Function Tests, M. Clin. North America **21**: 281, 1937.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

SULFAPYRIDINE, Effect of Sulfanilamide and, on Hemoglobin Metabolism and Hepatic Function, Watson, C. J., and Spink, W. W. Arch. Int. Med. 65: 825, 1940.

Sulfanilamide in customary therapeutic doses usually causes acceleration of the metabolism of hemoglobin characterized by an increase of urobilinogen in the feces and a varying increase in the reticulocyte percentage. The most marked acceleration of hemoglobin metabolism is represented by the unusual cases in which outspoken hemolytic anemia occurs. This condition is therefore to be regarded as a much more marked degree of a usual toxic effect of the drug. Limited data indicate that sulfapyridine has the same effect as sulfanilamide on hemoglobin metabolism.

The occurrence of macrocytic or normocytic, mildly hypochromic anemia after administration of sulfanilamide or sulfapyridine indicates a disturbance in hemoglobin formation in addition to increased hemolysis.

In many persons the administration of sulfanilamide in the usual doses is followed by some evidence of dysfunction of the liver, such as urobilinogenuria, elevation of the serum bilirubin, or outspoken jaundice. The last-mentioned condition is in part of the regurgitation type, as is evidenced by the frequent occurrence of a direct van den Bergh reaction. Sixteen cases of jaundice following sulfanilamide therapy have been encountered. But one instance has been noted in which jaundice followed use of sulfapyridine. Administration of sulfapyridine resulted in an elevation of the serum bilirubin in two cases in which frequent determinations were made before, during, and after administration of the drug. This is in contrast with the results in the group treated with sulfanilamide, in which significant elevation of the serum bilirubin was usual. Although the data on sulfapyridine are as yet too limited to permit definite conclusions, there is reason to believe that this drug may not be as disturbing to hepatic function as is sulfanilamide.

CARCINOMA, Cutaneous, Diagnosed Clinically Without an Autopsy. Result of Treatment in a Consecutive Series, Warren, S., Simmons, C. C., and Rea, S. L. J. A. M. A. **114:** 1619, 1940.

Of 829 treated carcinomas of the skin not verified by biopsy, 84 per cent were followed for five years. There were 57 per cent three-year cures and 48 per cent five-year cures of all the tumors treated. If the cases lost and dead of intercurrent disease are counted as cures, the five-year cures would be 84 per cent; if they are entirely excluded, the percentage would be 76. Recurrences occurred in 13 per cent of cases showing primary healing followed one year or more.

Primary healing occurred in 94 per cent of the lesions followed one year or more. More than one-fourth of the deaths from cutaneous carcinoma occurred after primary healing; primary healing should not be considered a criterion of cure.

Failures are due largely to the use of very light filtered radon applied to the surface in inadequate dosage. X-rays or radium used at a distance, as is the more general practice today, would have given greater depth doses and probably have given better results.

VULVA, Benign and Malignant Tumors of, Folsome, C. E. J. A. M. A. **114:** 1499, 1940.

Metaplasia and malignant change in apparently innocuous vulvar tumors are inadequately emphasized. Papillomas, sebaceous cysts, pigmented moles, leiomyofibromas, vulvar breast tissue, and sweat gland adenomas are some of the benign vulvar tumors which may, at a later date in a woman's life, degenerate to vulvar malignancy.

Treatment and care of the patient presenting herself with a vulvar cancer are tending to become more rational in the light of present anatomicopathologic knowledge of vulvar cancers. Vulval surgery combined with resection of superficial and deep inguinal lymph nodes, the so-called Basset technique, with modifications as outlined by Taussig, continues to be the best treatment for carcinoma of the vulva.

By stressing periodic pelvic examinations, cancer educational programs may materially assist in preventing cancer of the vulva. By paying attention to simple lesions and the benign tumors of the vulva, the physician may prevent carcinoma of the vulva.

COLLOIDAL GOLD, A Method of Preparation, Bartholomew, R. J., and Gent, N. L. Australian J. Exper. Biol. & M. Sc. **18**: 89, 1940.

A method is described for the preparation of colloidal gold sols for use in the Lange test by the reduction of gold chloride with sodium citrate.

A sol of correct sensitivity can be prepared (a) by using a suitable amount of citrate in the preparation, (b) by adding acid to an initially "hard" sol, (c) by adding alkali to an excessively sensitive sol, (d) by mixing suitable proportions of a "hard" sol and an excessively sensitive sol. Method (b) is preferred for routine use, and a technique for preparation by this method is described.

Not a single failure has been encountered in preparing citrate-reduced sols, and the sols have been found so uniform in behavior that it seems unnecessary to use a "paretic" fluid for standardization.

The method follows:

Into a liter flask put 675 ml. distilled water, close the flask with a ground-in reflux condenser, and bring the water to the boil over a fairly large flame. Remove the condenser and add 7 ml. of 1 per cent gold chloride solution followed by 17.5 ml. of 1 per cent sodium citrate solution. Replace the condenser and continue the boiling for fifteen minutes. Remove from the flame and allow to cool.

To one 50 ml. portion of the sol add nothing, and to three other 50 ml. portions add 0.05 ml., 0.10 ml., and 0.15 ml., respectively, of N/10 hydrochloric acid. Set up each mixture by the conventional technique against a known "paretic" cerebrospinal fluid and against a known "normal" fluid, then allow the mixtures to stand overnight. Select the mixture which gives the strongest reaction with the paretic fluid, yet does not give a reaction greater than 0 0 1 1 0 0 0 0 0 0 with the normal fluid, then add the corresponding quantity of acid to the main bulk of the sol, thoroughly agitating the mixture during the addition.

TYPHOID, Isolation of Paratyphoid, Dysentery and, From Feces and Urine, Ruys, A. C. Brit. M. J., April 13, p. 606, 1940.

This comparative study indicates that in cases of typhoid fever it is advisable to use the media of Wilson and Blair and of L. Muller; in paratyphoid B, those of Muller and Ruys; and in the enteric form of the disease, those of Kauffman and Ruys. Endo's agar should not be neglected because it may give a positive result one day earlier than any of the other media.

In bacillary dysentery both Leifson's desoxycholate-citrate agar and Endo's agar should be used.

SULFAPYRIDINE, Evaluation of the Clinical Toxicity of Sulfanilamide and, Brown, W. H., Thornton, W. B., and Wilson, J. S. J. A. M. A. **114**: 1606, 1940.

The vomiting caused by the administration of sulfapyridine appears to be of both local and central origin. Mucilage of tragacanth and nicotinic acid are of value in combating the vomiting and other mild toxic reactions.

Although methemoglobinemia contributes to cyanosis in a small number of cases, sulfhemoglobinemia has not been encountered.

The serious toxic manifestations have been found twice as frequently in the series treated by sulfapyridine as compared with the series on sulfanilamide.

Oliguria, hematuria, pain in the costovertebral angle, and anuria are serious and fairly common complications of intensive sulfapyridine therapy, particularly when the concentrations in the blood are high. These sequelae are related to the insolubility of acetylsulfapyridine, which precipitates in characteristic crystals in acid and alkaline urines. For this reason, the fluid intake should approximate 3,000 c.c. or more in twenty-four hours, and the urine volume in a like period should not be allowed to fall under 1,000 c.c.

Although leucopenia occurs more frequently in the sulfapyridine series and especially when the dosage is heavy, it is also encountered in both series and may occur when the doses are small or treatment is of short duration.

Acute hemolytic anemia with icterus was encountered only in the cases in which sulfanilamide was administered. Both series comprised instances of gradual and suddenly developing anemia.

Three cases of granulocytopenia were encountered in 100 cases in which sulfapyridine was given. All three occurred in cases in which treatment was maintained for longer than fifteen days at low blood concentrations.

Patients treated with intensive doses and in whom high blood concentrations of sulfapyridine were attained showed a very high incidence of serious toxic reactions.

Drug rashes and fevers are useful signs of toxicity, but serious toxic complications may occur without these warnings.

Sulfapyridine may remain in the liver in small quantities for as long as forty days after chemotherapy has been terminated. No conclusion has been reached as to whether sulfapyridine causes hepatitis.

Sulfapyridine is essentially more toxic than sulfanilamide.

GLUCOSE TOLERANCE, Reverse or "Paradoxical" Blood Sugar Response to Ingested Glucose, Wheelon, H. Endocrinology **26:** 743, 1940.

Laboratory and clinical findings are presented on 40 patients, each of whom showed a reverse or "paradoxical" blood sugar response to the ingestion of glucose, and a sugar-free urine.

The average fasting blood sugar level for this series was 140 mg., or a figure indicative of mild hyperglycemia. Blood sugar levels of 70 mg. or less were found twenty times on 10 patients during the glucose tolerance test. The initial blood sugar response of 14 glucose tolerance tests performed on 7 patients subsequent to the initial tests was an average elevation of 33 mg. above the fasting level, followed by a maximum depression of 19 mg. below the fasting reading. Five patients in the series of 7 initial tests developed hypoglycemic sugar levels ten times. A like number of patients developed hypoglycemic levels eight times in the fourteen subsequent series of tests. Two patients only, however, developed hypoglycemic levels in each of the two series.

Ninety-nine physical, and 248 chemical, a total of 347 determinations were made on the 40 patients in addition to those incident to the glucose tolerance test, urine and Wassermann examinations. A total of 39, or 40 per cent, of the 99 physical determinations lay beyond the limits of normal variation. A total of 107, or 43 per cent, of 248 chemical determinations lay outside the range of figures assumed to represent "normal variations."

Seventy-two clinical diagnoses, aside from those established by laboratory procedures, were made on the 40 patients. Diagnoses referable to disturbances of the nervous system were made on 29, or 73 per cent, of the 40 patients.

A correlation of the various findings shows that each of the 40 individuals studied had an average of 5.5 deviations from normal standards, in addition to the primary one, or reverse response to the ingestion of glucose.

It may be concluded that a rather large number of physical and chemical abnormal variations and diagnosable clinical states are found associated in various degrees with

reverse or "paradoxical" blood sugar responses to the ingestion of glucose. It may be concluded also that the medical management of patients showing reverse glucose responses, as demonstrated on 7 patients, results in the amelioration of the symptomatology and a return of the glucose response toward the usual normal.

SODIUM SULFAPYRIDINE, Use of, by Hypodermoclysis, Taplin, G. V., Jacox, R. F., and Howland, J. W. J. A. M. A. **114:** 1733, 1940.

Sodium sulfapyridine has been used by hypodermoclysis in more than 50 cases of pneumonia and numerous other conditions for which sulfapyridine was indicated but in which oral administration was difficult or impossible. The drug was given in from 0.3 to 0.7 per cent solution in physiologic solution of sodium chloride and no local reactions were observed in any of the cases. More than 1,100 Gm. of the drug have been given by this method.

This route of administration is advocated when the drug is not tolerated by mouth or is poorly absorbed from the gastrointestinal tract and when a high sustained concentration in the blood is imperative.

SULFANILAMIDE, Absorption of, as an Index of Blood Flow in the Intestine of Man, Stead, E. A., and Kunkel, P. Am. J. M. Sc. **199:** 681, 1940.

The blood flow in the human intestine has been investigated by determining the appearance time and the concentration curve in the blood of free sulfanilamide when a known amount of sulfanilamide in solution is introduced directly into the small intestine or colon.

Under basal conditions a blood level of 6 to 8 mg. per 100 c.c. of free sulfanilamide was obtained in ten to thirteen minutes when 300 c.c. of a 1 per cent solution of sulfanilamide were placed in the duodenum.

Heating the body or inducing collapse in an individual with postural hypotension did not alter the curve of sulfanilamide in the blood. This is interpreted as indicating that the basal blood flow to the small intestine was low and that the rapid appearance of sulfanilamide in the blood was the result of its rapid diffusion.

Under basal conditions sulfanilamide was absorbed much more slowly from the large bowel. When 300 c.c. of a 1 per cent solution of sulfanilamide were given rectally, the blood level rose to between 2.4 and 4 mg. per 100 c.c. at the end of one and one-half hours.

Pitressin failed to influence the rate of absorption of sulfanilamide when the substance was given by duodenal tube. Pitressin caused a delay in absorption when the sulfanilamide was placed in the large bowel.

The rapid absorption of the sulfanilamide in the collapse induced by the upright position in a person with postural hypotension suggested that even in shock sulfanilamide diffuses so rapidly that it is adequately absorbed once it is present in solution in the small intestine. Dilatation of the stomach and closure of the pylorus may, however, prevent the drug from reaching the small intestine.

SULFATHIAZOLE, Crystalline Concretions in the Renal Tubules Following, Pepper, D. S., and Horack, H. M. Am. J. M. Sc. **199:** 674, 1940.

Concretions obstructing the renal tubules and in the pelves and bladder were discovered at autopsy in a patient following treatment with sulfathiazole. On analysis the material was composed of a derivative of sulfathiazole. The gross and histologic findings in the kidneys were identical with those described by others as occurring in experimental animals. The probable etiologic factors influencing the site of the precipitation of sulfapyridine and sulfathiazole crystals are discussed. Because of the intrarenal precipitation of sulfathiazole, it is felt renal complications resulting from the use of this drug may be more serious than those following the use of sulfapyridine.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Electrocardiography*

THIS book is intended to serve as an introduction to the study of electrocardiography and is designed for the use of the general practitioner, the medical student, and others who are not primarily cardiologists. The use throughout the book of the standard nomenclature advocated by the American Heart Association is to be commended.

The book is an atlas of one hundred electrocardiographs with clinical diagnosis and interpretations. There is an interlarding of textual material. Although the authors state that the tracings have been carefully standardized, the reviewer would have preferred to have the standardizations given with each tracing, instead of being confined to the first. It is felt, however, that the aim of the authors is achieved very satisfactorily, and that the book may be recommended to the audience for which it was intended.

Vitamin E†

IN APRIL, 1939, a symposium was held on vitamin E in London. Taking part in this international, but predominantly British, discussion were many leaders in the field. The proceedings were edited by A. L. Bacharach and J. C. Drummond. The monograph constitutes a valuable summary of our knowledge in a highly important biochemical field.

The little book falls naturally into three subdivisions: the chemical structure and properties of tocopherol (vitamin E); the physiologic action of vitamin E and the consequences of vitamin E deficiency; and the clinical and veterinary uses of wheat germ oil and vitamin E preparations. In the eighteen years since the discovery of an antisterility vitamin its structural formula has been established and it has been synthesized. In addition to the biological estimation, there are four chemical methods for its analysis. The therapy of avitaminosis E is fast becoming rationalized. Although much work remains to be done before the last word has been said on vitamin E, this book brings together in a concise form the great deal that has already been accomplished.

---

*Electrocardiography. By Chauncey C. Maher, B.S., M.D., Assistant Professor of Medicine, Northwestern University and the Montgomery Ward Medical Clinics; Attending Internist and Chairman of Department of Medicine at the Cook County Hospital, Chicago, Ill.; and Paul H. Wosika, M.D., M.S., Instructor in Medicine, Northwestern University and the Montgomery Ward Medical Clinics; Attending Physician at Passavant Memorial Hospital; Associate in Medicine at the Cook County Hospital, Chicago, Ill. Cloth, ed. 3, 334 pages, $4.00. A William Wood Book. Williams & Wilkins Co., Baltimore, Md., 1940.

†Vitamin E. A symposium held under the auspices of the Food Group (Nutrition Panel) of the Society of Chemical Industry on April 22, 1939, at the School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.I. England. Cloth, 88 pages, $2.00. Chemical Publishing Co., Inc., New York, 1940.

CORRESPONDENCE

Wichita, Kansas,
October 9, 1940.

The Editor,
JOURNAL OF LABORATORY AND CLINICAL MEDICINE.
Dear Sir:

Perhaps some of the readers are tired of being unable to think while shaking a Kahn rack full of test tubes in their Kahn shaking machine. Perhaps, at the same time, they do not wish to throw away their Kahn racks and purchase the new rubber coated ones, which are silent in operation. Here is a way to silence the old racks.

A bottle of "Rug Sta," which is manufactured by the makers of Old English Wax, will do the trick. Merely take an old tooth brush and lay the "Rug Sta" on in a thick smooth coat. It is white when wet and colorless when dry. Two coats will usually suffice to make a silent test tube rack out of the noisiest of them.

JACK G. STEELE,
Midwest Laboratory.

Washington, D. C.
September 16, 1940.

The Editor,
JOURNAL OF LABORATORY AND CLINICAL MEDICINE.
Dear Sir:

Microfilm copying of the original articles referred to in this JOURNAL and published in the periodicals received by the Army Medical Library of Washington is being done by "Medicofilm Service," a non-profit organization operating in the Army Medical Library. The charge is 30 cents for each complete article not exceeding 30 pages in length and 10 cents for each additional 10 pages or fraction thereof. A circular of information and containing a list of the more than 4,000 periodicals received by the Library will be sent upon request to: Medicofilm Service.

Orders and remittances should be sent to:

Medicofilm Service, Army Medical Library,
7th St. & Independence Ave., S.W.,
Washington, D. C.

A. SEIDELL,
Medicofilm Service.